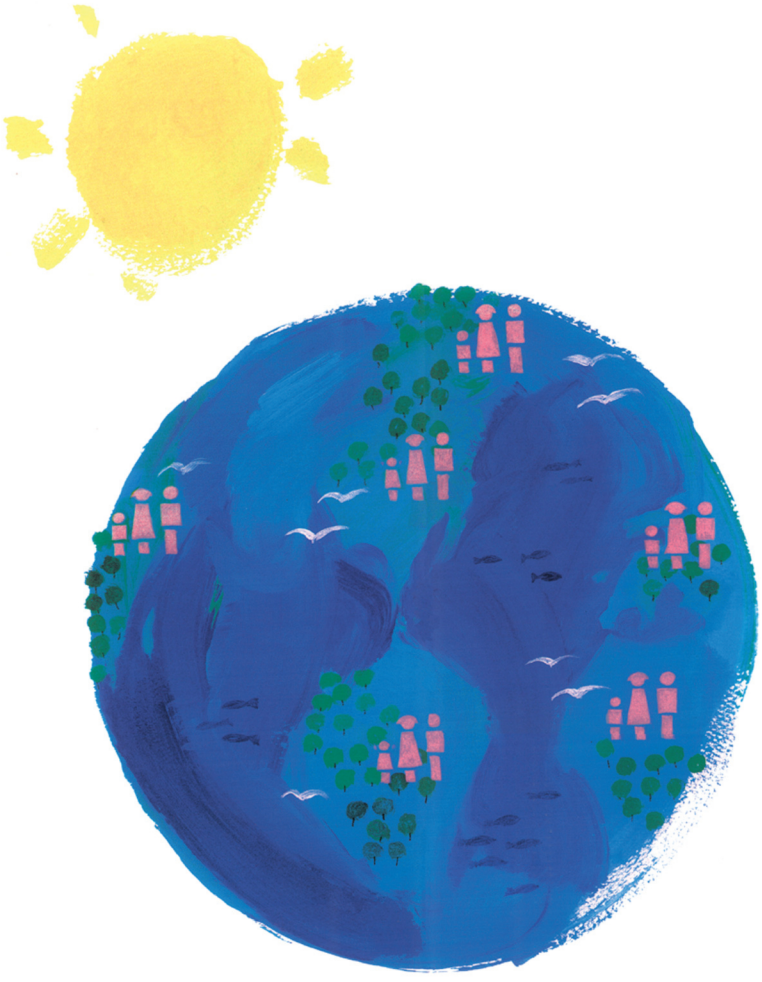
NIH Publication Number 10-7515



ICCVAM Test Method Evaluation Report: Recommendation to Discontinue Use of the Low Volume Eye Test for Ocular Safety Testing

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
Department of Health and Human Services

About the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

In 1997, the National Institute of Environmental Health Sciences (NIEHS), one of the National Institutes of Health, established ICCVAM to:

- Coordinate interagency technical reviews of new and revised toxicological test methods, including alternative test methods that reduce, refine, or replace the use of animals
- Coordinate cross-agency issues relating to validation, acceptance, and national and international harmonization of new, modified, and alternative toxicological test methods

On December 19, 2000, the ICCVAM Authorization Act (Public Law 106-545, 42 U.S.C. 285*l*-3) established ICCVAM as a permanent interagency committee of NIEHS under NICEATM.

ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability. ICCVAM promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety or hazards of chemicals and products and that reduce, refine (decrease or eliminate pain and distress), and/or replace animal use. NICEATM administers ICCVAM and provides scientific and operational support for ICCVAM-related activities. More information about NICEATM and ICCVAM can be found on the NICEATM-ICCVAM website (http://iccvam.niehs.nih.gov) or obtained by contacting NICEATM (telephone: [919] 541-2384, e-mail: niceatm@niehs.nih.gov).

ICCVAM is an interagency committee with representatives from the following 15 U.S. Federal regulatory and research member agencies that require, use, generate, or disseminate toxicological information:*

- *Consumer Product Safety Commission*
- *Department of Agriculture*
- *Department of Defense*
- *Department of Energy*
- Department of Health and Human Services
  - Centers for Disease Control and Prevention
    - *Agency for Toxic Substances and Disease Registry*
    - *National Institute of Occupational Safety and Health*
  - *Food and Drug Administration*
  - National Institutes of Health
    - *Office of the Director*
    - *National Cancer Institute*
    - *National Institute of Environmental Health Sciences*
    - *National Library of Medicine*
- *Department of the Interior*
- Department of Labor
  - *Occupational Safety and Health Administration*
- *Department of Transportation*
- *Environmental Protection Agency*

*Italics indicate those agencies represented on ICCVAM, as specified in the ICCVAM Authorization Act.

On the cover: The NICEATM-ICCVAM earth-and-sun graphic symbolizes the important role of new and alternative toxicological methods in protecting and advancing the health of people, animals, and our environment.

ICCVAM Test Method Evaluation Report: Recommendation to Discontinue Use of the Low Volume Eye Test for Ocular Safety Testing

Interagency Coordinating Committee on the Validation of Alternative Methods

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
Department of Health and Human Services

2010

NIH Publication No. 10-7515

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

This document is available electronically at

http://iccvam.niehs.nih.gov/methods/ocutox/LVET-TMER.htm

When referencing this document, please cite as follows:
ICCVAM. 2010. ICCVAM Test Method Evaluation Report: Recommendation to Discontinue Use of the Low Volume Eye Test for Ocular Safety Testing. NIH Publication No. 10-7515. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

Table of Contents

List of Tables

List of Abbreviations and Acronyms

ºC	Degrees centigrade
AHT	Animal health technologist
BRD	Background review document
CPSC	U.S. Consumer Product Safety Commission
CV	Coefficient of variation
ECVAM	European Centre for the Validation of Alternative Methods
EPA	U.S. Environmental Protection Agency
ESAC	European Centre for the Validation of Alternative Methods Scientific Advisory Committee
EU	European Union
FDA	U.S. Food and Drug Administration
FR	*Federal Register*
g	Gram
GHS	United Nations Globally Harmonized System of Classification and Labelling of Chemicals
GLP	Good Laboratory Practice
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ILS	Integrated Laboratory Systems, Inc.
IS	Irritation Score
JaCVAM	Japanese Center for the Validation of Alternative Methods
kg	Kilogram
LVET	Low volume eye test
MAS	Maximum average score
MeSH	Medical Subject Headings
mg	Milligram
mL	Milliliter
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NSAID	Nonsteroidal anti-inflammatory drug
NTP	U.S. National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OTWG	ICCVAM Ocular Toxicity Working Group
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
SC	Subcutaneous
SRD	Summary review document
TG	Test guideline
TSA	Test substance administration

UN	United Nations
U.S.	United States

Interagency Coordinating Committee on the Validation of Alternative Methods: Agency Representatives

Agency for Toxic Substances and Disease Registry
* Moiz Mumtaz, Ph.D.
Bruce Fowler, Ph.D.
Edward Murray, Ph.D.
Eric Sampson, Ph.D.

Consumer Product Safety Commission
* Marilyn L. Wind, Ph.D. (Chair)
+ Kristina Hatlelid, Ph.D.
Joanna Matheson, Ph.D.

Department of Agriculture
* Jodie Kulpa-Eddy, D.V.M. (Vice-Chair)
+ Elizabeth Goldentyer, D.V.M.

Department of Defense
* Robert E. Foster, Ph.D.
+ Patty Decot
Harry Salem, Ph.D.
Peter J. Schultheiss, D.V.M., DACLAM

Department of Energy
* Michael Kuperberg, Ph.D.
+ Marvin Stodolsky, Ph.D.

Department of the Interior
* Barnett A. Rattner, Ph.D.
+ Sarah Gerould, Ph.D. (to Feb. 2009)

Department of Transportation
* George Cushmac, Ph.D.
+ Steve Hwang, Ph.D.

Environmental Protection Agency
Office of Pesticide Programs
* John R. "Jack" Fowle III, Ph.D., DABT
+ Vicki Dellarco, Ph.D.
+ Tina Levine, Ph.D.
Deborah McCall
Christine Augustyniak, Ph.D. *(U.S. Coordinator, OECD Test Guidelines Program)*
Office of Pollution Prevention and Toxics
Jerry Smrchek, Ph.D. *(U.S. Coordinator, OECD Test Guidelines Program,* to July 2009*)*
Office of Research and Development
Suzanne McMaster, Ph.D. (to Dec. 2008)
Julian Preston, Ph.D. (to July 2009)
Stephanie Padilla, Ph.D. (to July 2009)
Office of Science Coordination and Policy
Karen Hamernik, Ph.D. (to July 2009)

Food and Drug Administration
Office of the Commissioner
* Suzanne Fitzpatrick, Ph.D., DABT
Center for Biologics Evaluation and Research
Richard McFarland, Ph.D., M.D.
Ying Huang, Ph.D.
Center for Devices and Radiological Health
Melvin E. Stratmeyer, Ph.D.
Vasant G. Malshet, Ph.D., DABT
Center for Drug Evaluation and Research
+ Abigail C. Jacobs, Ph.D.
Paul C. Brown, Ph.D.
Center for Food Safety and Applied Nutrition
David G. Hattan, Ph.D.
Robert L. Bronaugh, Ph.D.
Center for Veterinary Medicine
Devaraya Jagannath, Ph.D.
M. Cecilia Aguila, D.V.M.
National Center for Toxicological Research
Paul Howard, Ph.D.
Donna Mendrick, Ph.D.
William T. Allaben, Ph.D. (to Jan. 2009)
Office of Regulatory Affairs
Lawrence D'Hoostelaere, Ph.D.

National Cancer Institute
* T. Kevin Howcroft, Ph.D.
Chand Khanna, D.V.M., Ph.D.
Alan Poland, M.D. (to Oct. 2008)

National Institute of Environmental Health Sciences
* William S. Stokes, D.V.M., DACLAM
+ Raymond R. Tice, Ph.D.
Rajendra S. Chhabra, Ph.D., DABT
Jerrold J. Heindel, Ph.D.

National Institute for Occupational Safety and Health
* Paul Nicolaysen, V.M.D.
+ K. Murali Rao, M.D., Ph.D.

National Institutes of Health
* Margaret D. Snyder, Ph.D.

National Library of Medicine
* Pertti (Bert) Hakkinen, Ph.D.
+ Jeanne Goshorn, M.S.

Occupational Safety and Health Administration
* Surender Ahir, Ph.D.

* Principal agency representative
+ Alternate principal agency representative

Acknowledgements

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Ocular Toxicity Working Group (OTWG)

U.S. Consumer Product Safety Commission
Adrienne Layton, Ph.D.
Marilyn L. Wind, Ph.D.

U.S. Department of Defense
Harry Salem, Ph.D.

U.S. Department of Transportation
Steve Hwang, Ph.D.

U.S. Environmental Protection Agency
Office of Pesticide Programs
Meta Bonner, Ph.D.
Jonathan Chen, Ph.D.
John R. "Jack" Fowle III, Ph.D., DABT
Masih Hashim, D.V.M., Ph.D.
Karen Hicks
Marianne Lewis
Debbie McCall
Timothy McMahon, Ph.D.
Mark Perry
John Redden
Jenny Tao, Ph.D.
Office of Research and Development
Andrew Geller, Ph.D.
Office of Science Coordination and Policy
Karen Hamernik, Ph.D.

U.S. Food and Drug Administration
Center for Drug Evaluation and Research
Paul Brown, Ph.D.
Wiley Chambers, M.D.
Abigail (Abby) Jacobs, Ph.D.
Jill Merrill, Ph.D., DABT (OTWG Chair)
Center for Food Safety and Applied Nutrition
Robert Bronaugh, Ph.D.
Donnie Lowther
Office of the Commissioner
Suzanne Fitzpatrick, Ph.D., DABT

National Institute Environmental Health Sciences
Mark F. Cesta, D.V.M, DACVP
Raymond (Buck) Grissom, Ph.D.
William Stokes, D.V.M., DACLAM

Occupational Safety and Health Administration
Surender Ahir, Ph.D.

European Centre for the Validation of Alternative Methods – Liaison
João Barroso, Ph.D.
Thomas Cole, Ph.D.
Valerie Zuang, Ph.D.

Japanese Center for the Validation of Alternative Methods – Liaison
Hajime Kojima, Ph.D.

Alternative Ocular Safety Testing Methods and Approaches Independent Scientific Peer Review Panel (May 19-21, 2009)

Hongshik Ahn, Ph.D.
Professor
Stony Brook University
Stony Brook, NY

Paul T. Bailey, Ph.D.
Bailey & Associates Consulting
Neshanic Station, NJ

Richard Dubielzig, D.V.M.
Professor
School of Veterinary Medicine
University of Wisconsin–Madison
Madison, WI

Henry Edelhauser, Ph.D.[1]
Professor of Ophthalmology and Director of Ophthalmic Research
Emory University School of Medicine
Atlanta, GA

Mark Evans, D.V.M., Ph.D., DACVP
Pathology Lead for Ophthalmology Therapeutic Area
Pfizer Global Research and Development
La Jolla Drug Safety Research and Development
San Diego, CA

A. Wallace Hayes, Ph.D., DABT, FATS, ERT
Visiting Scientist (Harvard)
Principal Advisor
Spherix Incorporated
Bethesda, MD
Harvard School of Public Health
Andover MA

James V. Jester, Ph.D.
Professor of Ophthalmology and Biomedical Engineering
Endowed Chair
University of California–Irvine
Orange, CA

Tadashi Kosaka, D.V.M., Ph.D.
Associate Director
Chief, Laboratory of Immunotoxicology and Acute Toxicology
Toxicology Division
The Institute of Environmental Toxicology
Ibaraki, Japan

Alison McLaughlin, MSc, DABT
Health Canada
Environmental Impact Initiative
Office of Science and Risk Management
Health Products and Food Branch
Ottawa, Ontario
Canada

J. Lynn Palmer, Ph.D.
Associate Professor
Dept. of Palliative Care & Rehabilitation Medicine
University of Texas M.D. Anderson Cancer Center
Houston, TX

Robert Peiffer, Jr., D.V.M., Ph.D., DACVO
Senior Investigator
Merck Research Laboratories
Safety Assessment Toxicology
Doylestown, PA

Denise Rodeheaver, Ph.D., DABT
Assistant Director
Alcon Research Ltd.
Dept. of Toxicology
Fort Worth, TX

Donald Sawyer, D.V.M., Ph.D., DACVA
Professor Emeritus
Retired, Michigan State University
(Summer Residence)
Okemos MI
(Winter Residence)
Tucson, AZ

[1] Drs. Edelhauser, Thake, and Tseng were unable to attend the public meeting on May 19–21, 2009. However, they were involved in the peer review of the background review documents and concur with the conclusions and recommendations included in the *Independent Scientific Peer Review Panel Report – Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Strategies.*

Kirk Tarlo, Ph.D., DABT
Scientific Director
Comparative Biology and Safety Sciences
Amgen, Inc.
One Amgen Center Drive
Thousand Oaks, CA

Daryl C. Thake, D.V.M., DACVP[1]
Midwest ToxPath Sciences Inc.
Chesterfield, MO

Scheffer Tseng, M.D., Ph.D.[1]
Director, Ocular Surface (OS) Center
Medical Director OS Research & Education Foundation
Director R&D Department
Tissue Tech, Inc.
Ocular Surface Center, P.A.
Miami, FL

Jan van der Valk, Ph.D.
Senior Scientist
Departments of Animals, Science and Society
Faculty of Veterinary Medicine
Utrecht University
Netherlands Centre Alternatives to Animal Use (NCA)
Utrecht, Netherlands

Philippe Vanparys, Ph.D., DABT
Managing Director
Cardam, Centre for Advanced Research & Development
Mol, Belgium

Philippe Vanparys, Ph.D., DABT
Managing Director
Cardam, Centre for Advanced Research & Development
Mol, Belgium

Maria Pilar Vinardell, Ph.D.
Director, Department of Physiology
Professor of Physiology and Pathology
Department Fisologia
Facultat de Farmacia
Universitat de Barcelona
Barcelona, Spain

Fu-Shin Yu, Ph.D.
Director of Research
Department of Ophthalmology & Anatomy
School of Medicine
Wayne State University
Detroit, MI

Sherry Ward, Ph.D., MBA
In Vitro Toxicology Consultant
BioTred Solutions
Science Advisor
International Foundation for Ethical Research (IFER)
New Market, MD

Daniel Wilson, Ph.D., DABT
Mammalian Toxicology Consultant
Toxicology and Environmental Research Consulting
The Dow Chemical Co.
Midland, MI

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

National Institute of Environmental Health Sciences

William Stokes, D.V.M., DACLAM
Director; Project Officer

Deborah McCarley
Special Assistant; Assistant Project Officer

NICEATM Support Contract Staff (Integrated Laboratory Systems [ILS], Inc.)

David Allen, Ph.D.
Jonathan Hamm, Ph.D.
Nelson Johnson
Brett Jones, Ph.D.
Elizabeth Lipscomb, Ph.D.
Linda Litchfield
Steven Morefield, M.D.
Catherine Sprankle
James Truax, M.A.
Linda Wilson

Statistical Consultant for ILS, Inc.

Joseph Haseman, Ph.D.

Other Acknowledgements

ICCVAM and NICEATM gratefully acknowledge the following individuals and institutions that submitted data to NICEATM for the evaluation of alternative ocular safety testing methods and approaches.

Access Business Group
Luann Potts
Tom Truszkowski
Washington, DC

Clorox Company
Pleasanton, CA

Colgate-Palmolive Company
Piscataway, NJ

Cosmetics, Toiletry, and Fragrance Association
Carol Eisenmann, Ph.D.
Washington, DC

The Dial Corporation
Scottsdale, AZ

ECOLabs
St. Paul, MN

ECVAM
Chantra Eskes, Ph.D.
Ispra, Italy

ExxonMobil Biomedical Sciences, Inc.
James Freeman, Ph.D.
Annandale, NJ

Institute for In Vitro Sciences, Inc.
Rodger Curren, Ph.D.
John Harbell, Ph.D. (to March 2006)
Jennifer Nash, M.S.
Angela Sizemore, B.S.
Gaithersburg, MD

JohnsonDiversey, Inc.
John Hamilton, Ph.D.
Sarah Willems
Sturdivant, WI

Johnson & Johnson Pharmaceutical R&D
Phillipe Vanparys, Ph.D.
Freddy van Goethem, Ph.D.
Beerse, Belgium

L'Oreal
Christine Van den Berghe, Ph.D.
Paris, France

MatTek Corporation
Patrick Hayden, Ph.D.
Ashland. MA

Merck
Joseph Sina, Ph.D.
West Point, PA

National Institute of Health Sciences
Yasuo Ohno, Ph.D.
Tokyo, Japan

S.C. Johnson & Son
Nicole Cuellar, M.S.
Judith Swanson, B.S./B.A.
Racine, WI

The Procter & Gamble Company
Dan Marsman, D.V.M., Ph.D., DABT
Len Sauers, Ph.D.
Cincinnati, OH

TNO Nutrition and Food Research Institute
Menk Prinsen, Ph.D.
Zeist, The Netherlands

U.S. Food and Drug Administration
Donnie Lowther
College Park, MD

ZEBET
Manfred Liebsch, Ph.D.
Horst Spielmann, Dr. med.
Berlin, Germany

This page intentionally left blank

Preface

Eye injury is a leading cause of visual impairment in the United States with 40,000 to 50,000 new cases of impaired vision reported each year.[1] Many eye injuries occur due to contact with workplace or household products or chemicals. Accidents involving common household products (e.g., oven cleaner and bleach) cause about 125,000 eye injuries each year.[2] These products often result in chemical burns and emergency room visits.[3] Each day about 2,000 U.S. workers have a job-related eye injury that requires medical treatment. Although the majority of these eye injuries result from mechanical sources, chemical burns from industrial chemicals or cleaning products are common.[4]

To prevent eye injuries, regulatory agencies require testing to determine if chemicals and products may cause eye damage. This testing information is used to classify the ocular hazard and determine appropriate labeling to warn consumers and workers of the potential hazard. Appropriate labeling tells users how to avoid exposure that could damage the eye and what emergency procedures should be followed if there is accidental exposure. Nearly all ocular safety testing has been conducted using the Draize rabbit eye test, although *in vitro* methods can now be used to identify whether substances cause severe irritation or permanent eye damage. The Draize rabbit eye test (Draize et al. 1944) involves instillation of 0.1 mL of the test substance into the conjunctival sac of one eye. The other eye serves as the untreated control. The eye is examined at least daily for up to 21 days. The presence and severity of any injuries to the cornea, conjunctiva, and the iris (tissues inside the eye) are scored and the duration that the injuries persist is recorded.

More recently, Griffith et al. (1980) developed the low volume eye test (LVET) with the intention that it would more accurately reflect the human response, since the traditional Draize rabbit eye test was considered to consistently overpredict the human ocular hazard potential. The LVET differs from the Draize rabbit eye test in that only 10% of the volume used in the Draize is applied to the eye (10 μL vs. 100 μL), and the test substance is applied directly on the center of the cornea instead of in the conjunctival sac.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently reviewed the validity of the LVET as a replacement for the Draize rabbit eye test. This was necessary because LVET data were used to support the validity of a proposed non-animal *in vitro* testing strategy for antimicrobial cleaning products. As a part of this evaluation, ICCVAM and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) requested the submission of data and information on substances tested in rabbits using the LVET protocol (73 FR 18535).[5]

ICCVAM carefully compiled and assessed all available data and arranged an independent scientific peer review. ICCVAM and the Ocular Toxicity Working Group (OTWG) solicited and considered public comments and stakeholder involvement throughout the evaluation process. As part of their ongoing collaboration with ICCVAM, scientists from the European Centre for the Validation of Alternative Methods (ECVAM) and the Japanese Center for the Validation of Alternative Methods (JaCVAM) served as liaisons to the OTWG. ICCVAM, NICEATM, and the OTWG prepared a draft summary review document (SRD) describing the validation status of the LVET, including its reliability and accuracy, and draft test method recommendations for its usefulness and limitations. ICCVAM released this document to the public for comment on March 31, 2009. ICCVAM also

---

[1] Available at http://www.preventblindness.org/resources/factsheets/Eye_Injuries_FS93.pdf
[2] Available at http://www.geteyesmart.org/eyesmart/injuries/home.cfm
[3] From the CPSC NEISS Database, 2007
[4] Available at http://www.cdc.gov/niosh/topics/eye/
[5] Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-E8-6969.pdf

announced a meeting of the independent international scientific peer review panel (Panel) (74 FR 14556).[6]

The Panel met in public session on May 19–21, 2009, to review the ICCVAM draft SRD for completeness and accuracy. The Panel then evaluated (1) the extent to which the draft SRD addressed established validation and acceptance criteria and (2) the extent to which the draft SRD supported ICCVAM's draft test method recommendations. Before concluding their deliberations, the Panel considered written comments and comments made at the meeting by public stakeholders.

ICCVAM provided the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) with the LVET draft SRD and draft test method recommendations, a summary of the conclusions and recommendations from the Panel meeting, and all public comments for discussion at their meeting on June 25–26, 2009, where public stakeholders were given another opportunity to comment. A detailed timeline of the evaluation is included with this report.

ICCVAM solicited and considered public comments and stakeholder involvement throughout the test method evaluation process. ICCVAM considered the SACATM comments, the conclusions of the Panel, and all public comments before finalizing the ICCVAM test method recommendations. The recommendations and the SRD, which is provided as an appendix to this report, are incorporated in this ICCVAM test method evaluation report. As required by the ICCVAM Authorization Act, ICCVAM will forward its recommendations to U.S. Federal agencies for consideration. Federal agencies must respond to ICCVAM within 180 days after receiving the ICCVAM test method recommendations. ICCVAM recommendations are available to the public on the NICEATM–ICCVAM website.[7] Agency responses will also be made available on the website as they are received.

We gratefully acknowledge the many individuals who contributed to the preparation, review, and revision of this report. We especially recognize the Panel members for their thoughtful evaluations and generous contributions of time and effort. Special thanks are extended to Dr. A. Wallace Hayes for serving as the Panel Chair and to Dr. Paul Bailey, Dr. Donald Sawyer, Dr. Kirk Tarlo, and Dr. Daniel Wilson for their service as Evaluation Group Chairs. We thank the OTWG for assuring a meaningful and comprehensive review. We especially thank Dr. Jill Merrill (U.S. Food and Drug Administration Center for Drug Evaluation and Research) and Dr. Karen Hamernik (U.S. Environmental Protection Agency, until April 2009) for serving as Co-Chairs of the OTWG. Integrated Laboratory Systems, Inc., the NICEATM support contractor, provided excellent scientific support, for which we thank Dr. David Allen, Dr. Jonathan Hamm, Nelson Johnson, Dr. Brett Jones, Dr. Elizabeth Lipscomb, and James Truax. Finally, we thank the European Centre for the Validation of Alternative Methods liaisons Dr. João Barroso, Dr. Thomas Cole, and Dr. Valerie Zuang and the Japanese Center for the Validation of Alternative Methods liaison Dr. Hajime Kojima for their participation and contributions.

Marilyn Wind, Ph.D.
Deputy Associate Executive Director
Directorate for Health Sciences
U.S. Consumer Product Safety Commission
Chair, ICCVAM

William S. Stokes, D.V.M., DACLAM
Rear Admiral/Assistant Surgeon General, U.S. Public Health Service
Director, NICEATM
Executive Director, ICCVAM

---

[6] Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/E9-7220.pdf
[7] Available at http:// iccvam.niehs.nih.gov/methods/ocutox/AMCP.htm

Executive Summary

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently evaluated the validation status of the *in vivo* low volume eye test (LVET). This test method evaluation report provides ICCVAM's recommendations on the usefulness and limitations of the LVET as an alternative to the Draize rabbit eye test (Draize et al. 1944) for assessing substances' ocular irritation potential.

The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, ICCVAM, and its Ocular Toxicity Working Group prepared a summary review document (SRD). The SRD, which summarizes the current validation status of the LVET, is based on published studies and forms the basis for draft ICCVAM test method recommendations. The draft SRD and ICCVAM recommendations were provided to an independent international scientific peer review panel (Panel) and to the public for comment. A detailed timeline of the ICCVAM evaluation process is appended to this report.

The Panel met in public session on May 19–21, 2009, to discuss its peer review of the ICCVAM draft SRD. The Panel members discussed how well the information contained in the draft SRD supported ICCVAM's draft test method recommendations. In finalizing this test method evaluation report and the SRD, which is included as an appendix, ICCVAM considered (1) the conclusions and recommendations of the Panel, (2) comments from ICCVAM's Scientific Advisory Committee on Alternative Toxicological Methods, and (3) public comments.

Specific ICCVAM Test Method Recommendations

Test Method Usefulness and Limitations

ICCVAM does not consider the LVET a valid replacement for the Draize rabbit eye test. Accordingly, ICCVAM does not recommend the LVET for prospective ocular safety testing. If animals must be used for ocular safety testing, ICCVAM recommends using the modified Draize rabbit eye test protocol that incorporates the recommended topical anesthetics, systemic analgesics, and humane endpoints. However, ICCVAM concluded that retrospective LVET data can be used in a weight-of-evidence approach to classify ocular hazards provided that the validity of each type of evidence used for such assessments is adequately characterized.[8]

ICCVAM recommends using Draize data to select reference chemicals for all future validation studies of new, revised, and alternative test methods for ocular safety testing. Priority should be given to chemicals for which there are both Draize data and human data (e.g., from accidental exposures or standardized ethical human studies).

Test Method Protocol

As indicated above, ICCVAM does not recommend any future testing using the LVET and therefore does not recommend a test method protocol.

Future Studies

ICCVAM recommends that additional requests be made for available historical data that participating companies may have on the LVET (e.g., in-house or external studies they have supported, or research and testing studies). Where such data are available, efforts should be made to determine (1) which could be used in a weight-of-evidence approach and (2) how they might be considered.

---

[8] The ECVAM Scientific Advisory Committee (ESAC) does not consider the LVET a valid replacement for the Draize rabbit eye test. ESAC also concludes that retrospective LVET data can be used in a weight-of-evidence approach to classify ocular hazards (ESAC 2009; **Appendix D**).

This page intentionally left blank

1.0 Introduction

The low volume eye test (LVET) is an *in vivo* rabbit eye test that, like the Draize test, was designed to determine the extent of potential ocular hazard of a test substance. Both tests evaluate the ocular irritation response when a single dose of a test substance is applied to the eye of a rabbit. Developed by Griffith et al. (1980), the LVET differs from the Draize rabbit eye test primarily by applying 10 μL of a test substance directly on the cornea instead of 100 μL in the conjunctival sac. Scoring of corneal, iridal, and conjunctival lesions in the LVET is identical to that in the Draize rabbit eye test.

To date, the LVET has not been demonstrated as an adequately valid *in vivo* reference test method. It has not been formally accepted by any regulatory agency as a stand-alone test for ocular safety testing. The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently reviewed the usefulness and limitations of the LVET as a proposed replacement for ocular safety testing, because LVET data were used to support the validity of an *in vitro* testing strategy for antimicrobial cleaning products.

The ICCVAM Authorization Act of 2000 (Public Law 106-545, 42 United States Code 285*l*-3) charged ICCVAM with coordinating the technical evaluation of new, revised, and alternative test methods that have regulatory applicability. The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) administers ICCVAM and provides scientific support for ICCVAM activities.

NICEATM works with the ICCVAM Ocular Toxicity Working Group (OTWG) to evaluate alternative methods and testing strategies. Drs. João Barroso, Tom Cole, and Valerie Zuang represented the European Centre for the Validation of Alternative Methods (ECVAM), and Dr. Hajime Kojima was the liaison from the Japanese Center for the Validation of Alternative Methods (JaCVAM) to the OTWG.

To facilitate the peer review, the OTWG and NICEATM prepared a draft summary review document (SRD) on the use of the LVET in ocular toxicity testing. The document provided information and data from published and unpublished data. A background review document for the LVET was originally submitted to ECVAM. However, the companies that provided unpublished data for the document would not agree to its release. Therefore, the data included in the ECVAM background review document are not considered here.

In April 2008, NICEATM and ICCVAM published a *Federal Register* notice requesting the submission of data and information on substances tested in rabbits using the LVET protocol (73 FR 18535).[1] The notice also requested nominations for an independent expert peer review panel (Panel). These requests were also disseminated via the ICCVAM electronic mailing list and through direct requests to over 100 stakeholders. No data were received in response to the request; however, 12 individuals or organizations submitted comments. Twenty potential panelists were nominated for consideration (see **Section 4.0**).

The SRD forms the basis for the ICCVAM test method recommendations described in this test method evaluation report. The ECVAM and JaCVAM liaisons to the OTWG provided input and contributed throughout the evaluation process. Detailed timelines of the ICCVAM evaluation and the development of the final SRD for the LVET method are provided as **Appendices A** and **B**, respectively.

On March 31, 2009, ICCVAM announced the availability of the ICCVAM draft documents. The *Federal Register* notice also announced a public Panel meeting (74 FR 14556[2]) to review the

[1] Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-E8-6969.pdf
[2] Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/E9-7220.pdf

validation status of the LVET test method and several other proposed alternatives for ocular safety testing, The ICCVAM draft SRD and draft test method recommendations were provided to the Panel and posted on the NICEATM–ICCVAM website, along with all public comments received before the Panel meeting.

The Panel met in public session from May 19–21, 2009, to review the completeness and accuracy of the ICCVAM draft SRD. The Panel then evaluated (1) the extent to which the draft SRD addressed established validation and acceptance criteria and (2) the extent to which the draft SRD supported ICCVAM's draft test method recommendations. Interested stakeholders from the public commented at the Panel meeting. The Panel considered all comments before concluding their deliberations. On July 12, 2009, ICCVAM posted the final report of the Panel's recommendations (see **Appendix C**) on the NICEATM–ICCVAM website for public review and comment (announced in 74 FR 33444).[3]

ICCVAM gave the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) the draft SRD, draft test method recommendations, the Panel report, and all public comments. SACATM discussed the information at their meeting on June 25–26, 2009; and public stakeholders were given another opportunity to comment.

ICCVAM and the OTWG considered the SACATM comments, the Panel report, and all public comments when finalizing this test method evaluation report and the accompanying SRD (**Appendix B**). As required by the ICCVAM Authorization Act, ICCVAM will make this test method evaluation report and the final LVET SRD available to the public and to U.S. Federal agencies for consideration. Federal agencies must respond to ICCVAM within 180 days after receiving ICCVAM test method recommendations. Agency responses will be posted on the NICEATM–ICCVAM website as they are received.

[3] Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/E9-16388.pdf

2.0 ICCVAM Recommendations for the LVET Test Method

2.1 ICCVAM Recommendations: Test Method Usefulness and Limitations

ICCVAM does not consider the LVET a complete replacement for the Draize rabbit eye test and therefore does not recommend the LVET for prospective ocular safety testing. If animals must be used in ocular safety testing, ICCVAM recommends that the Draize rabbit eye test be used as recommended with topical anesthetics, systemic analgesics, and humane endpoints (ICCVAM 2010). However, ICCVAM concluded that retrospective LVET data can be used in a weight-of-evidence approach to identify potential ocular irritants.[4] ICCVAM also recommends that the selection of reference chemicals for validation of alternative ocular toxicity test methods be based on Draize data, not on LVET data.

Independent Peer Review Panel Conclusions and Recommendations

The Panel concluded that, in the absence of all available data, including a background review document (BRD) prepared by ECVAM, they could not make definitive conclusions or recommendations on the validation status of the LVET.

2.2 ICCVAM Recommendations: Test Method Protocol for the LVET Test Method

As indicated above, ICCVAM does not recommend prospective testing with the LVET and therefore does not recommend a specific test method protocol.

Independent Peer Review Panel Conclusions and Recommendations

As noted above, the Panel could not make definitive conclusions and recommendations on the LVET test method.

2.3 ICCVAM Recommendations: Future Studies for the LVET Test Method

ICCVAM recommends that further inquires be made about the existence of any additional historical data that participating companies have on the LVET (e.g., research and testing studies, or in-house or external studies they have supported). Where such data are available, efforts should be made to determine which data could be used in a weight-of-evidence approach and how it might be considered.

Independent Peer Review Panel Conclusions and Recommendations

The Panel emphasized the need to further inquire about the existence of any additional historical data the participating companies have on the LVET (e.g., in-house or external studies they have supported).

[4] The ECVAM Scientific Advisory Committee (ESAC) does not consider the LVET a valid replacement for the Draize rabbit eye test. ESAC also concludes that retrospective LVET data can be used in a weight-of-evidence approach to classify ocular hazards (ESAC 2009; **Appendix D**).

3.0 Validation Status of the LVET Test Method

ICCVAM reviewed the validity of the LVET because LVET data is used to support the validity of one of the *in vitro* test methods proposed in the *in vitro* testing strategy for antimicrobial cleaning products. The accuracy of the LVET was compared to that of the Draize rabbit eye test and to available human data and experience. A BRD for the LVET was originally submitted to ECVAM, but the companies that provided unpublished data for the document would not agree to its release. In addition, the ECVAM BRD does not include additional reference data for severe irritants tested in both the LVET and the Draize test. Consequently, it provides no additional data to evaluate the accuracy of the LVET compared to the Draize rabbit eye test for severe irritants. Therefore, the data included in the ECVAM background review document are not considered here.

The LVET is an *in vivo* rabbit eye test developed by Griffith et al. (1980). Like the Draize rabbit eye test, the LVET was designed to determine the extent of a test substance's potential ocular hazard. It evaluates the irritation response when a single dose of the test substance is administered to the eye of a rabbit. The LVET differs from the Draize rabbit eye test primarily by applying 10 μL of a test substance directly on the cornea instead of 100 μL applied in the conjunctival sac. Scoring of corneal, iridal, and conjunctival lesions in the LVET is identical to that in the Draize rabbit eye test.

Most publicly available LVET data represent only limited types (i.e., surfactant-containing personal care and household cleaning products) and numbers of substances. The same is true for traditional Draize rabbit data with which to compare and evaluate the accuracy of the LVET. Available human data (clinical studies and accidental exposures) proposed to support the accuracy of the LVET are largely with mild irritants or nonirritating substances, as are the corresponding LVET data. These substances are predominantly surfactant-containing cosmetic and personal care product formulations.

Ethical considerations have limited the types of substances that can be tested in human clinical studies. As a result, LVET comparisons to human clinical study data are based on tests with mild irritants or substances not labeled as irritants. Such data provide little assurance to the regulatory agencies charged with protecting public health that the LVET can provide adequate protection from substances that may cause moderate or severe ocular injuries in humans.

Accidental exposures are generally not considered a reliable source of information on true ocular hazard potential. Eyes are likely flushed with large volumes of water immediately after accidental exposure. They may not represent the most severe lesion that might be produced by such an exposure. Accidental exposures do not allow definitive quantitative measures of amount and time of exposure needed for human reference data. Some consumer products (e.g., bleach) that cause corrosive ocular lesions in humans at certain concentrations have not been tested in the LVET at comparable concentrations. The LVET is proposed as more likely to approximate the volume of a substance that could enter the human eye experimentally; however, there are limited data to indicate whether it can accurately identify the ocular hazard of substances known to cause moderate, severe, or permanent human ocular injuries.

In contrast, there are no documented instances in which a substance that produced a severe irritant/corrosive response in humans was not also classified as a severe irritant/corrosive in the Draize rabbit eye test.

4.0 ICCVAM Consideration of Public and SACATM Comments

The ICCVAM evaluation process provides numerous opportunities for stakeholder involvement. The public may submit written comments and provide oral comments at ICCVAM independent peer review panel meetings and SACATM meetings. **Table 4-1** lists the nine opportunities for public comments during the ICCVAM evaluation of the validation status of alternative ocular safety testing methods and approaches. The number of public comments received in response to each of the opportunities is also indicated. Thirty-seven comments were submitted. Comments received in response to or related to the *Federal Register* notices are accessible on the NICEATM–ICCVAM website.[5] The following sections, delineated by *Federal Register* notice, briefly discuss the public comments received.

Table 4-1 Opportunities for Public Comment

Opportunities for Public Comment	Date	Number of Public Comments Received
70 FR 13512: Request for Data on Non-Animal Methods and Approaches for Determining Skin and Eye Irritation Potential of Antimicrobial Cleaning Product Formulations; Request for Nominations for an Independent Expert Panel	March 21, 2005	0
72 FR 26396: Request for Data on the Use of Topical Anesthetics and Systemic Analgesics for *In Vivo* Eye Irritation Testing	May 9, 2007	1
72 FR 31582: Request for Ocular Irritancy Test Data From Human, Rabbit, and *In Vitro* Studies Using Standardized Testing Methods	June 7, 2007	0
73 FR 18535: Non-Animal Methods and Approach for Evaluating Eye Irritation Potential for Antimicrobial Cleaning Products (AMCPs): Request for Nominations for an Independent Expert Panel and Submission of Relevant Data	April 4, 2008	12
74 FR 14556: Announcement of an Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods; Availability of Draft Background Review Documents (BRD); Request for Comments	March 31, 2009	8
74 FR 19562: Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)	April 29, 2009	2
Independent Scientific Peer Review Panel Meeting: Alternative Ocular Safety Testing Methods	May 19–21, 2009	12
SACATM Meeting, Arlington Hilton, Arlington, VA	June 25–26, 2009	2
74 FR 33444: Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches; Notice of Availability and Request for Public Comments	July 13, 2009	0

[5] Available at http://ntp-apps.niehs.nih.gov.iccvambp/searchPubCom.cfm

4.1 Public Comments in Response to 70 FR 13512 (March 21, 2005): Request for Data on Non-Animal Methods and Approaches for Determining Skin and Eye Irritation Potential of Antimicrobial Cleaning Product Formulations; Request for Nominations for an Independent Expert Panel

NICEATM requested (1) submission of data that would assist in evaluating the validation status of non-animal methods and approaches used for determining the skin and eye irritation potential of AMCP formulations to meet regulatory hazard classification and labeling purposes and (2) nominations of expert scientists to serve as members of an independent peer review panel.

No data or nominations were received in response to this *Federal Register* notice.

4.2 Public Comments in Response to 72 FR 26396 (May 9, 2007): Request for Data on the Use of Topical Anesthetics and Systemic Analgesics for *In Vivo* Eye Irritation Testing

NICEATM requested submission of (1) data and information on the use of topical anesthetics and systemic analgesics for alleviating pain and distress in rabbits during eye irritation testing and (2) information about other procedures and strategies that may reduce or eliminate pain and distress associated with *in vivo* eye irritation methods.

NICEATM received no public comments relevant to the LVET test method.

4.3 Public Comments in Response to 72 FR 31582 (June 7, 2007): Request for Ocular Irritancy Test Data From Human, Rabbit, and *In Vitro* Studies Using Standardized Testing Methods

NICEATM requested data on substances tested for ocular irritancy in humans, rabbits, and/or *in vitro* to be used to:

- Review the state of the science in regard to the availability of accurate and reliable *in vitro* test methods for assessing the range of potential ocular irritation activity, including whether ocular damage is reversible or not
- Expand NICEATM's high-quality ocular toxicity database. *In vitro* test methods for which data are sought include but are not limited to (1) the bovine corneal opacity and permeability test, (2) the isolated rabbit eye test, (3) the isolated chicken eye test, and (4) the hen's egg test–chorioallantoic membrane

No data or information was received in response to this *Federal Register* notice.

4.4 Public Comments in Response to 73 FR 18535 (April 4, 2008): Non-Animal Methods and Approach for Evaluating Eye Irritation Potential for Antimicrobial Cleaning Products (AMCPs): Request for Nominations for an Independent Expert Panel and Submission of Relevant Data

NICEATM requested the following:

- Nominations of expert scientists to serve as members of an independent peer review panel
- Submission of relevant data and information on AMCPs or related substances obtained from (1) human testing or experience, including reports from accidental exposures, and (2) rabbit testing using the standard eye test or the LVET

- *In vitro* ocular irritation test methods such as the bovine corneal opacity and permeability test method, the Cytosensor® Microphysiometer test method, and the EpiOcular test method, including data supporting the accuracy and reproducibility of these methods

In response to this *Federal Register* notice, NICEATM received 12 comments, including nominations of 20 potential panelists. The nominees were included in the database of experts from which the Panel was selected. No additional data were received.

4.5 Public Comments in Response to 74 FR 14556 (March 31, 2009): Announcement of an Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods; Availability of Draft Background Review Documents; Request for Comments

NICEATM requested public comments on the draft BRDs, SRDs, and draft ICCVAM test method recommendations that were provided to an independent scientific peer review panel meeting (May 19–21, 2009). These documents summarized the current validation status of several test methods and testing strategies for identifying potential ocular irritants. The test methods and testing strategies included the following:

- A testing strategy that proposes the use of three *in vitro* test methods to assess the eye irritation potential of AMCPs
- Four *in vitro* test methods for identifying moderate (EPA Category II, UN Globally Harmonized System of Classification and Labelling of Chemicals [GHS] Category 2A) and mild (EPA Category III, GHS Category 2B) ocular irritants and substances not classified as ocular irritants (EPA Category IV, GHS Not Classified)
- The *in vivo* LVET
- A proposal for the routine use of topical anesthetics, systemic analgesics, and humane endpoints to avoid and minimize pain and distress during *in vivo* ocular irritation testing

NICEATM received 20 comments in response to this *Federal Register* notice. Eight written comments were received before the Panel meeting, and 12 oral comments were provided at the Panel meeting.

Public Responses, Written

Two written comments were relevant to the LVET test method.

Comment:

One commenter provided additional information and references for the use of LVET data as *in vivo* reference data. The commenter's main points were that (1) personal care and surfactant-based cleaning products do not result in eye injuries observed in people, (2) accidental human exposure data should be included in the assessment of eye irritation, and (3) both the sensitivity and specificity of the LVET should be evaluated. The commenter also provided additional data on the performance of known human corrosives in the LVET and comments on the analysis of data in Gettings et al. (1996, 1998).

ICCVAM Response:

The additional data and references were provided to the Panel before its public meeting and are included in the LVET final summary review document (**Appendix B**). ICCVAM considers human experience data to be important for consideration in a weight-of-evidence approach to hazard categorization.

Comment:

One commenter provided additional information and references on the historical LVET database to support use of the LVET as an *in vivo* reference test method. The commenter's main points follow:

- The historical LVET database includes known human ocular corrosives and a range of substances from different chemical classes and hazard categories.
- Several historical parallel LVET–Draize datasets are available and include a range of substances from different hazard categories.
- The Draize test is subject to inherent variability.
- Both the LVET and the Draize overpredict the human response, but the LVET is more representative of the human response than the Draize test.
- Human experience data are an important source of data that should be considered in a weight-of-evidence approach.
- The choice of 10 μL as the dose volume for LVET is supported by anatomical/physiological considerations between rabbits and humans.

ICCVAM Response:
ICCVAM does not consider the LVET a valid replacement for the Draize rabbit eye test. ICCVAM does not recommend the LVET for prospective ocular safety testing. ICCVAM also concluded that retrospective LVET data can be used in a weight-of-evidence approach to identify potential ocular irritants, provided that there is adequate characterization of the validity of each type of evidence used for such weight-of-evidence assessments.[6]

Public Responses, Oral
Twelve oral public comments were provided at the Panel meeting. Three comments remarked specifically on the LVET test method.

Comment:
One commenter stated that eye irritation testing is done to protect the public and that accidental exposure data should be included in the evaluation.
ICCVAM Response:
While it is important to consider accidental exposure data in a weight-of-evidence approach to hazard categorization, accidental exposures are generally not considered a reliable source of information on true ocular hazard potential because of the uncertain concentration and volume of the substance.

Comment:
Two commenters indicated that the LVET is being discussed because it was used as an *in vivo* reference test method for some of the data provided for the AMCP testing strategy. The commenters stated that only LVET data exist for many of the AMCPs, and these data were used to determine the prediction model to support registration of these AMCPs. The LVET test method is no longer used, but there are historical data that can and should be used.
ICCVAM Response:
Most publicly available LVET data represent only limited types and numbers of substances (i.e., surfactant-containing personal care and household cleaning products). The same is true for traditional Draize rabbit data with which to compare and evaluate the accuracy of the LVET. The available comparative LVET and human (clinical studies and accidental exposures) data proposed to support its accuracy are largely with substances that are mild irritants or nonirritating. These substances are predominantly surfactant-containing cosmetic and personal care product formulations.

[6] ESAC does not consider the LVET a valid replacement for the Draize rabbit eye test. ESAC also concludes that retrospective LVET data can be used in a weight-of-evidence approach to classify ocular hazards (ESAC 2009; **Appendix D**).

4.6 Public Comments in Response to 74 FR 19562 (April 29, 2009): Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

NICEATM announced the SACATM meeting (June 25–26, 2009) and requested written and public oral comments on the agenda topics.

Public Response:
NICEATM received four comments in response. Two written comments were received before the meeting, and two oral comments were provided at the SACATM meeting.

NICEATM received no public comments relevant to the LVET test method.

SACATM Response:
In general, SACATM was pleased with the Panel report. One SACATM member expressed the need for harmonization in the assessment of performance standards. Another SACATM member said the focus should be on the GHS system because it will ultimately be adopted. Another SACATM member expressed concern regarding the availability of the Cytosensor Microphysiometer.

4.7 Public Comments in Response to 74 FR 33444 (July 13, 2009): Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches; Notice of Availability and Request for Public Comment

NICEATM requested submission of written public comments on the independent scientific peer review panel report. No public comments were received.

5.0 References

Draize J, Woodard G, Calvery H. 1944. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 82:377-390.

Gettings SD, Lordo RA, Demetrulias J, Feder PI, Hintze KL. 1996. Comparison of low-volume, Draize and *in vitro* eye irritation test data. I. Hydroalcoholic formulations. Food Chem Toxicol 34:737–749.

Gettings SD, Lordo RA, Feder PI, Hintze KL. 1998. A comparison of low volume, Draize and *in vitro* eye irritation test data. III. Surfactant-based formulations. Food Chem Toxicol 36:209–231.

Griffith J, Nixon G, Bruce R, Reer P, Bannan E. 1980. Dose-response studies with chemical irritants in the albino rabbit eye as a basis for selecting optimum testing conditions for predicting hazard to the human eye. Toxic Appl Pharmacol 55:501–513.

ICCVAM. 2010. ICCVAM Test Method Evaluation Report on Routine Use of Topical Anesthetics, Systemic Analgesics, and Humane Endpoints to Avoid or Minimize Pain and Distress in Ocular Safety Testing. NIH Publication No. 10-7514. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

Appendix A

ICCVAM Evaluation Timeline

This page intentionally left blank

ICCVAM Evaluation Timeline

December 27, 2007	Background Review Document titled In Vitro Approach for EPA Toxicity Labeling of AMCPs received from the Institute for In Vitro Science, Inc. (IIVS).
April 4, 2008	*Federal Register* Notice (73 FR 18535) – Non-Animal Methods and Approaches for Evaluating Eye Irritation Potential for Antimicrobial Cleaning Products (AMCPs): Request for Nominations for an Independent Expert Panel and Submission of Relevant Data.
March 31, 2009	*Federal Register* Notice (74 FR 14556) – Announcement of an Independent Scientific Peer Review Panel Meeting on the Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches; Availability of Draft Background Review Documents (BRD) and Summary Review Documents (SRD); Request for Comments.
May 19-21, 2009	Independent Scientific Peer Review Panel holds a public meeting, with opportunity for public comments, at CPSC Headquarters in Bethesda, MD. The Panel was charged with reviewing the current validation status of alternative ocular safety testing methods and strategies, and commenting on the extent to which the information in the draft BRD and SRD supported the draft ICCVAM test method recommendations.
June 25-26, 2009	SACATM public meeting, SACATM and public comments on the draft Panel conclusions and recommendations.
July 13, 2009	*Federal Register* Notice (74 FR 33444) – Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches: Notice of Availability and Request for Public Comments.
October 29, 2009	ICCVAM endorses the Test Method Evaluation Report, which includes the final Background Review Document and Summary Review Document.

This page intentionally left blank

Appendix B

ICCVAM Summary Review Document: The Low Volume Eye Test

This page intentionally left blank

ICCVAM Summary Review Document:

The Low Volume Eye Test

Interagency Coordinating Committee on the Validation of Alternative Methods

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
Department of Health and Human Services

2010

This page intentionally left blank

Table of Contents

List of Tables

List of Abbreviations and Acronyms

A.I.S.E.	International Association for Soaps, Detergents and Maintenance Products
ATSDR	Agency for Toxic Substances and Disease Registry
BRD	Background review document
CASRN	Chemical Abstracts Service Registry Number
Conj	Conjunctiva
CPSC	(U.S.) Consumer Product Safety Commission
CR	Conjunctival redness
DER	Data Evaluation Report
EC	European Commission
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EC/HO	European Commission/British Home Office
ECVAM	European Centre for the Validation of Alternative Methods
EEC	European Economic Community
EI	Extremely irritating
EPA	(U.S.) Environmental Protection Agency
EU	European Union
FDA	(U.S.) Food and Drug Administration
FR	*Federal Register*
FHSA	U.S. Federal Hazardous Substances Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GLP	Good Laboratory Practices
hr	Hour
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
JaCVAM	Japanese Center for the Validation of Alternative Methods
LVET	Low volume eye test
µL	Microliter
MAS	Maximum average score
MeSH	(National Library of Medicine) Medical Subject Headings
MI	Maximally irritating
Minim	Minimally irritating
MMAS	Mean maximum average score
MSDS	Material Safety Data Sheet
NA	Not applicable
NI	Nonirritating
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute of Occupational Safety and Health
NP	Not provided
NT	Not tested
NTP	(U.S.) National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OPPTS	Office of Prevention, Pesticides and Toxic Substances
OSHA	Occupational Safety and Health Administration
OTWG	Ocular Toxicity Working Group
PNI	Practically nonirritating
PSB	Product Safety Branch
scs	Test substance dosed on the superior conjunctival sac
SD	Standard deviation
TSCA	Toxic Substances Control Act
TG	Test Guideline
UN	United Nations

Interagency Coordinating Committee on the Validation of Alternative Methods: Agency Representatives

Agency for Toxic Substances and Disease Registry
* Moiz Mumtaz, Ph.D.
Bruce Fowler, Ph.D.
Edward Murray, Ph.D.
Eric Sampson, Ph.D.

Consumer Product Safety Commission
* Marilyn L. Wind, Ph.D. (Chair)
+ Kristina Hatlelid, Ph.D.
Joanna Matheson, Ph.D.

Department of Agriculture
* Jodie Kulpa-Eddy, D.V.M. (Vice-Chair)
+ Elizabeth Goldentyer, D.V.M.

Department of Defense
* Robert E. Foster, Ph.D.
+ Patty Decot
Harry Salem, Ph.D.
Peter J. Schultheiss, D.V.M., DACLAM

Department of Energy
* Michael Kuperberg, Ph.D.
+ Marvin Stodolsky, Ph.D.

Department of the Interior
* Barnett A. Rattner, Ph.D.
+ Sarah Gerould, Ph.D. (to Feb. 2009)

Department of Transportation
* George Cushmac, Ph.D.
+ Steve Hwang, Ph.D.

Environmental Protection Agency
Office of Pesticide Programs
* John R. "Jack" Fowle III, Ph.D., DABT
+ Vicki Dellarco, Ph.D.
+ Tina Levine, Ph.D.
Deborah McCall
Christine Augustyniak, Ph.D. *(U.S. Coordinator, OECD Test Guidelines Program)*
Office of Pollution Prevention and Toxics
Jerry Smrchek, Ph.D. *(U.S. Coordinator, OECD Test Guidelines Program,* to July 2009*)*
Office of Research and Development
Suzanne McMaster, Ph.D. (to Dec. 2008)
Julian Preston, Ph.D. (to July 2009)
Stephanie Padilla, Ph.D. (to July 2009)
Office of Science Coordination and Policy
Karen Hamernik, Ph.D. (to July 2009)

* Principal agency representative
+ Alternate principal agency representative

Food and Drug Administration
Office of the Commissioner
* Suzanne Fitzpatrick, Ph.D., DABT
Center for Biologics Evaluation and Research
Richard McFarland, Ph.D., M.D.
Ying Huang, Ph.D.
Center for Devices and Radiological Health
Melvin E. Stratmeyer, Ph.D.
Vasant G. Malshet, Ph.D., DABT
Center for Drug Evaluation and Research
+ Abigail C. Jacobs, Ph.D.
Paul C. Brown, Ph.D.
Center for Food Safety and Applied Nutrition
David G. Hattan, Ph.D.
Robert L. Bronaugh, Ph.D.
Center for Veterinary Medicine
Devaraya Jagannath, Ph.D.
M. Cecilia Aguila, D.V.M.
National Center for Toxicological Research
Paul Howard, Ph.D.
Donna Mendrick, Ph.D.
William T. Allaben, Ph.D. (to Jan. 2009)
Office of Regulatory Affairs
Lawrence D'Hoostelaere, Ph.D.

National Cancer Institute
* T. Kevin Howcroft, Ph.D.
Chand Khanna, D.V.M., Ph.D.
Alan Poland, M.D. (to Oct. 2008)

National Institute of Environmental Health Sciences
* William S. Stokes, D.V.M., DACLAM
+ Raymond R. Tice, Ph.D.
Rajendra S. Chhabra, Ph.D., DABT
Jerrold J. Heindel, Ph.D.

National Institute for Occupational Safety and Health
* Paul Nicolaysen, V.M.D.
+ K. Murali Rao, M.D., Ph.D.

National Institutes of Health
* Margaret D. Snyder, Ph.D.

National Library of Medicine
* Pertti (Bert) Hakkinen, Ph.D.
+ Jeanne Goshorn, M.S.

Occupational Safety and Health Administration
* Surender Ahir, Ph.D.

Acknowledgements

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Ocular Toxicity Working Group (OTWG)

U.S. Consumer Product Safety Commission
Marilyn L. Wind, Ph.D.
Adrienne Layton, Ph.D.

U.S. Department of Defense
Harry Salem, Ph.D.

U.S. Department of Transportation
Steve Hwang, Ph.D.

U.S. Environmental Protection Agency
Office of Pesticide Programs
Meta Bonner, Ph.D.
Jonathan Chen, Ph.D.
John R. "Jack" Fowle III, Ph.D., DABT
Masih Hashim, D.V.M., Ph.D.
Karen Hicks
Marianne Lewis
Debbie McCall
Timothy McMahon, Ph.D.
Mark Perry
John Redden
Jenny Tao, Ph.D.
Office of Research and Development
Andrew Geller, Ph.D.
Office of Science Coordination and Policy
Karen Hamernik, Ph.D.

U.S. Food and Drug Administration
Center for Drug Evaluation and Research
Paul Brown, Ph.D.
Wiley Chambers, M.D.
Abigail (Abby) Jacobs, Ph.D.
Jill Merrill, Ph.D., DABT (OTWG Chair)
Center for Food Safety and Applied Nutrition
Robert Bronaugh, Ph.D.
Donnie Lowther
Office of the Commissioner
Suzanne Fitzpatrick, Ph.D., DABT

National Institute of Environmental Health Sciences
Warren Casey, Ph.D., DABT
Mark F. Cesta, D.V.M, DACVP
Raymond (Buck) Grissom, Ph.D.
William Stokes, D.V.M., DACLAM

Occupational Safety and Health Administration
Surender Ahir, Ph.D.

European Centre for the Validation of Alternative Methods – Liaison
João Barroso, Ph.D.
Thomas Cole, Ph.D.
Valerie Zuang, Ph.D.

Japanese Center for the Validation of Alternative Methods – Liaison
Hajime Kojima, Ph.D.

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

National Institute of Environmental Health Sciences

William Stokes, D.V.M., DACLAM
Director; Project Officer

Warren Casey, Ph.D., DABT
Deputy Director

Deborah McCarley
Special Assistant; Assistant Project Officer

NICEATM Support Contract Staff (Integrated Laboratory Systems [ILS], Inc.)

David Allen, Ph.D.

Jonathan Hamm, Ph.D.

Nelson Johnson

Brett Jones, Ph.D.

Elizabeth Lipscomb, Ph.D.

Linda Litchfield

Steven Morefield, M.D.

Catherine Sprankle

James Truax, M.A.

Linda Wilson

Statistical Consultant for ILS, Inc.

Joseph Haseman, Ph.D.

This page intentionally left blank

Preface

Accidental contact with hazardous chemicals frequently causes eye injury and visual impairment. United States and international regulatory agencies currently use the Draize rabbit eye test (Draize et al. 1944) to identify potential ocular hazards associated with chemicals. The U.S. Consumer Product Safety Commission, U.S. Environmental Protection Agency, U.S. Food and Drug Administration, and U.S. Occupational Health and Safety Administration have testing regulations and/or guidelines and recommendations for assessing the ocular irritation potential of substances such as pesticides, household products, pharmaceuticals, cosmetics, and agricultural and industrial chemicals.

Although ocular safety assessment has clearly helped to protect consumers and workers, concerns have been raised about the humane aspects of the Draize rabbit eye test. Regulatory authorities have adopted various modifications that reduce the number of animals used and the potential pain and distress associated with the procedure. Significant progress has been made during the last decade. Now only one to three rabbits are required per test, compared to six rabbits in the original protocol. Provisions have been added that allow for animals with severe lesions or discomfort to be humanely euthanized.

The low volume eye test (LVET) was developed by Griffith et al. (1980) with the intent of refining the Draize rabbit eye test to reduce overlabeling of commercial products and more closely predict the human accidental response to ocular hazard. The Draize test was refined by applying the test substance to the corneal surface rather than to the conjunctival sac and by reducing the volume of exposure from 100 µL to 10 µL. However, the hypothesis that the LVET more closely predicts the human response than the Draize test for a wide applicability domain of test substances has not been clearly demonstrated yet. Thus the LVET has yet to be adopted as a reference test method by any regulatory agency.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) reviewed the validity of the LVET because LVET data was used to support the validity of a test method described in the *ICCVAM Test Method Evaluation Report: Current Validation Status of a Proposed* In Vitro *Testing Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products* (ICCVAM 2010). The ICCVAM Ocular Toxicity Working Group and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) have prepared this draft summary review document to summarize the current validation status of the LVET based on available information and data obtained by NICEATM. This draft summary review document forms the basis for draft ICCVAM test method recommendations, which are provided in a separate document.

An independent international scientific peer review panel met in public forum on May 19–21, 2009, to develop conclusions and recommendations for the LVET. The Panel included expert scientists nominated by the European Centre for the Validation of Alternative Methods and the Japanese Center for the Validation of Alternative Methods. We anticipate that these organizations will be able to use the Panel's independent report for their deliberations and development of test method recommendations. The Panel considered this summary review document and evaluated the extent to which the available information supported the draft ICCVAM test method recommendations. ICCVAM considered the conclusions and recommendations of the Panel, along with comments received from the public and the Scientific Advisory Committee on Alternative Toxicological Methods, before finalizing the summary review document and test method recommendations. These will be forwarded to Federal agencies for their consideration and acceptance decisions where appropriate.

We gratefully acknowledge the organizations and scientists who provided data and information for this document. We also acknowledge the efforts of those individuals who helped prepare this

summary review document, including the following staff from the NICEATM support contractor, Integrated Laboratory Systems, Inc.: David Allen, Jon Hamm, Nelson Johnson, Elizabeth Lipscomb, Brett Jones, Linda Litchfield, Gregory Moyer, Catherine Sprankle, and James Truax. We also thank the members of the ICCVAM Ocular Toxicity Working Group, chaired by Karen Hamernik, Ph.D. (EPA), and Jill Merrill, Ph.D. (U.S. Food and Drug Administration), and ICCVAM representatives who reviewed and provided comments throughout the process leading to this draft version. We also want to thank Valerie Zuang, Ph.D., and Dr. Hajime Kojima, Ph.D., the Ocular Toxicity Working Group liaisons from the European Centre for the Validation of Alternative Methods and the Japanese Center for the Validation of Alternative Methods, respectively, for their participation.

Marilyn Wind, Ph.D.
Deputy Associate Executive Director
Directorate for Health Sciences
U.S. Consumer Product Safety Commission
Chair, ICCVAM

William S. Stokes, D.V.M., DACLAM
Rear Admiral/Assistant Surgeon General, U.S. Public Health Service
Director, NICEATM
Executive Director, ICCVAM

Executive Summary

Accidental eye injury due to contact with hazardous chemicals is a major cause of visual impairment. United States and international regulatory agencies currently use the Draize rabbit eye test (Draize et al. 1944) to identify potential ocular hazards associated with chemicals. In the Draize rabbit eye test, 100 μL of the test substance is introduced into the conjunctival sac of each animal's eye. Alternatives to the Draize test have been explored to reduce the possibility of pain and distress during the test procedure.

Griffith et al. (1980) developed the low volume eye test (LVET) to both refine the rabbit eye test and more closely predict the human response to ocular hazard. In the LVET, the test substance is applied to the corneal surface rather than the conjunctival sac. The volume of exposure is decreased from 100 μL to 10 μL. However, the LVET has not been shown to predict the human response more closely than the Draize test for a wide array of test substances. Thus, the LVET has not yet been adopted as a reference test method by any regulatory agency. This report reviews available scientific literature and summarizes the usefulness and limitations of the LVET as an acceptable *in vivo* reference test method.

Most available LVET data were generated with surfactant-based mixtures or products, which produce only a mild ocular irritant response or no response. Gettings et al. (1996a) evaluated 25 surfactant formulations and their hazard classifications by the Environmental Protection Agency and Globally Harmonized System of Classification and Labelling of Chemicals. The authors reported several instances in which the LVET underpredicted an ocular corrosive or severe irritant response identified in the Draize test. While some claim that these data show the Draize test to be excessively overpredictive, there is limited information on the performance of known human corrosives in the LVET.

Freeberg et al. (1984) conducted both the LVET and the Draize test on 29 household cleaning products for which human accidental exposure data are available. The authors concluded that the LVET more accurately predicts the human accidental response to such substances. Similarly, Freeberg et al. (1986b) tested 14 cleaning products with both the LVET and Draize tests and compared the responses to human accidental eye exposures. They concluded that the LVET response corresponds more closely to the human experience than does the Draize rabbit eye test.

Ghassemi et al. (1993) and Roggeband et al. (2000) concluded that the smaller volume used in the LVET (10 μL) is more appropriate when compared directly with human clinical data. However, the lack of available Draize test data in these studies precludes any direct comparison with the LVET.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) reviewed the validity of the LVET because LVET data was used to support the validity of a test method described in the *ICCVAM Test Method Evaluation Report: Current Validation Status of a Proposed* In Vitro *Testing Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products* (ICCVAM 2010). LVET data are available for only limited types and numbers of substances (i.e., surfactant-containing personal and household cleaning products), precluding comprehensive evaluation of LVET performance.

Comparative human data from clinical studies and accidental exposures have been proposed to support the accuracy of the LVET. However, these data are primarily for mild or nonirritating substances. Ethical considerations have limited the severity of substances that can be tested in human clinical studies. As a result, LVET comparisons to human clinical study data are based on tests with mild irritants or substances not labeled as irritants. Regulatory agencies charged with protecting public health cannot be assured that the LVET can adequately protect against substances that may cause moderate or severe ocular injuries in humans.

The LVET may approximate experimentally the volume of a substance that could enter the human eye accidentally, but there are limited data to indicate whether it can accurately identify the ocular hazard of substances known to cause moderate, severe, or permanent human ocular injuries. In contrast, there are no documented instances in which a substance that produced a severe irritant/corrosive response in humans was not also classified as a severe irritant/corrosive in the Draize rabbit eye test.

1.0 Background on Ocular Safety Testing

Accidental eye injury is a leading cause of visual impairment in the United States. Many of these injuries occur due to contact with workplace or household chemicals. According to the National Institute of Occupational Safety and Health (NIOSH), each day about 2,000 U.S. workers have a job-related eye injury that requires medical treatment. Additional eye injuries occur in the home, with about 125,000 eye injuries a year caused by accidents involving common household products such as oven cleaner and bleach (source, American Academy of Ophthalmology). U.S. regulatory agencies such as the Consumer Product Safety Commission (CPSC), Environmental Protection Agency (EPA), Food and Drug Administration (FDA), and Occupational Safety and Health Administration (OSHA) have testing regulations and/or guidelines and recommendations to assess the hazard potential of substances that may come in contact with human eyes.

These testing requirements have effectively protected consumers and workers from potential eye injury (Wagoner 1997; Chiou 1999; McGwin et al. 2006). The primary method currently accepted by U.S. and international regulatory agencies for assessing ocular safety hazards is the Draize rabbit eye test (Draize et al. 1944). Testing guidelines describing the procedure have been published (EPA OPPTS 870.2400 [EPA 1998]), Organisation for Economic Co-operation and Development Test Guideline 405 [OECD 2002]) and several legislative statutes have been enacted that enable government agencies to regulate a variety of substances with the potential to pose a risk to ocular health and safety (see **Table 1-1**).

Table 1-1 Summary of Current U.S. Legislation Related to Ocular Health

Legislation (Year of Initial Enactment)	Agency	Substance
Food, Drug, and Cosmetic Act (1938)	Food and Drug Administration	Pharmaceuticals and cosmetics
Federal Insecticide, Fungicide, and Rodenticide Act (1947) and Federal Environmental Pesticide Control Act (1972)	Environmental Protection Agency	Pesticides
Federal Hazardous Substances Act (1964)	Consumer Product Safety Commission	Household products
Federal Hazardous Substances Act (1964) and Toxic Substances Control Act (1976)	Department of Agriculture and Environmental Protection Agency	Agricultural and industrial chemicals
Occupational Safety and Health Act (1970)	Occupational Safety and Health Administration	Occupational materials
Clean Air Act Amendments (1990)	Chemical Safety and Hazard Investigation Board and Environmental Protection Agency	Accidentally released chemicals and air pollutants

Adapted from Wilhelmus (2001).

2.0 Regulatory Testing Requirements for Ocular Hazards

The classification of irritant responses evaluated by each regulatory agency varies depending on their legislative mandate and specific goals for protecting human health (**Table 2-1)**. The EPA ocular irritation classification regulation and testing guidelines (EPA 1998, 2003) are based on the most severe response in one animal in a group of three or more animals. This classification system takes into consideration the kinds of ocular effects produced, as well as the reversibility and severity of the effects. The EPA classifies substances in ocular irritant Categories I through IV (EPA 2003). Category I substances are defined as corrosive or severe irritants, while classification from II to IV is based on decreasing severity of irritation and time required for irritation to clear. Irritation that clears in 8 to 21 days is classified as Category II, while irritation that clears within 7 days is classified as Category III. For Category IV substances, irritation clears within 24 hours.

The U.S. Federal Hazardous Substances Act (FHSA) guideline for ocular irritation classification (CPSC 1995) categorizes a test substance as corrosive, irritant, or substance not labeled as irritant. A corrosive, according to the FHSA, is a substance that causes visible destruction or irreversible alterations in the tissue at the site of contact (CPSC 1995). FHSA classification depends on the number of test animals that exhibit a positive ocular response within 72 hours after application of the test substance in the conjunctival sac.

For the purpose of harmonizing the classification of ocular irritants internationally, the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN 2007) includes two harmonized categories. One designates irreversible effects on the eye/serious damage to the eye (Category 1), and one designates reversible effects on the eye (Category 2). *Reversible effects* are further classified based on the duration of persistence. Category 2A (irritating to eyes) reverses within 21 days, and Category 2B (mildly irritating to eyes) reverses within 7 days. The GHS categories are based on severity of the lesions and/or the duration of persistence.

Hazard classification of ocular irritants in the European Union is characterized by two risk phrases: (1) R36 denotes “irritating to eyes”; (2) R41 denotes “risk of serious damage to the eyes” (EU 2001). These risk phrases are based on whether the levels of damage, averaged across the 24-, 48- and 72-hour observation times for each ocular lesion, fall within or above certain ranges of scores.

Table 2-1 Ocular Toxicity Classification Systems

Regulatory Agency (Authorizing Act)	Number of Animals	Observation Days (after treatment)	Mean score taken?	Positive Response	Classification Criteria
U.S. CPSC (Federal Hazardous Substances Act) OSHA (Occupational Safety and Health Act)	6 (12, 18 possible)	1, 2, 3	No	Opacity or Iritis ≥1 or Redness or Chemosis ≥2 for any animal on any day	1st Tier: 4 or more positive animals = Irritant 2–3 positive animals = Go to 2nd Tier 2nd Tier 3 or more positive animals = Irritant 1–2 positive animals = Go to 3rd Tier 3rd Tier : 1 positive animal = Irritant
U.S. EPA (FIFRA, Federal Environmental Pesticide Control Act, and TSCA)	At least 3	1 hr, 1, 2, 3, 7, 21	No	–Maximum score in an animal used for classification –Opacity or Iritis ≥1 or Redness or Chemosis ≥2	– One or more positive animals needed for classification in categories below. Category: I = Corrosive, corneal involvement, or irritation persisting more than 21 days II = Corneal involvement or irritation clearing in 8–21 days III = Corneal involvement or irritation clearing in 7 days or less IV = Minimal effects clearing in less than 24 hours **Definition of Full Reversal:** Opacity and Iritis scores = 0 and Redness and Chemosis scores ≤1

continued

Table 2-1 Ocular Toxicity Classification Systems (continued)

Regulatory Agency (Authorizing Act)	Number of Animals	Observation Days (after treatment)	Mean score taken?	Positive Response	Classification Criteria
European Union	1 if severe effects are suspected or 3 if no severe effects are suspected	1, 2, 3 (observation until Day 21)	Yes	Mean study values (scores of all animals in study averaged over Days 1, 2, and 3) of: Opacity or Chemosis ≥2, Redness ≥2.5, or Iritis ≥ 1 **OR** Individual animal mean values (scores for each endpoint are averaged for each animal over Days 1, 2, and 3) of: Opacity or Chemosis ≥2, Redness ≥ 2.5, or Iritis ≥ 1	**R36 Classification** (3) Mean study value where: 2 ≤ Opacity < 3 or 1 ≤ Iritis < 1.5 or Redness ≥2.5 or Chemosis ≥2 (2) If 2/3 tested animals have individual animal mean values that falls into one of the following categories: 2 ≤ Opacity <3 1 ≤ Iritis <2 Redness ≥2.5 Chemosis ≥2 **R41 Classification** (3)Mean study value where: Opacity ≥3 or Iritis >1.5 (2) If 2/3 tested animals have individual animal mean values that fall into one of the following categories: Opacity ≥3 Iritis = 2 (3) At least one animal (at the end of the observation period, typically Day 21) where Opacity or Chemosis ≥2, Redness ≥2.5 or Iritis ≥1
GHS: Irreversible Eye Effects	3	1, 2, 3 (observation until Day 21)	Yes	Mean animal values (over Days 1, 2, and 3) of: Opacity ≥3 and/or Iritis ≥1.5	–At least 2 positive response animals = Eye Irritant Category 1 –At least 1 animal with an Opacity, Iritis, Redness, or Chemosis score >0 on Day 21 = Eye Irritant Category 1 **Definition of Full Reversal:** Opacity, Iritis, Redness, and Chemosis scores = 0

continued

Table 2-1 Ocular Toxicity Classification Systems (continued)

Regulatory Agency (Authorizing Act)	Number of Animals	Observation Days (after treatment)	Mean score taken?	Positive Response	Classification Criteria
GHS: Reversible Eye Effects	3	1, 2, 3 (observation until Day 21)	Yes	Mean animal values (over Days 1, 2, and 3) of: Opacity or Iritis ≥1 or Redness or Chemosis ≥2 and the effect fully reverses in 7 or 21 days	–At least 2 positive response animals and the effect fully reverses in 21 days = Eye Irritant Category 2A –At least 2 positive response animals and effect fully reverses in 7 days = Eye Irritant Category 2B **Definition of Full Reversal:** Opacity, Iritis, Redness, and Chemosis scores = 0

Abbreviations: CPSC = U.S. Consumer Product Safety Commission; EPA = U.S. Environmental Protection Agency; FDA = U.S. Food and Drug Administration; FIFRA = Federal Insecticide, Fungicide, and Rodenticide Act; GHS = United Nations (UN) Globally Harmonized System of Classification and Labelling of Chemicals; OSHA = U.S. Occupational Safety and Health Administration; TSCA = Toxic Substances Control Act.

3.0 Principle of the Low Volume Eye Test

The low volume eye test (LVET) is an *in vivo* rabbit eye test that, like the Draize test, was designed to determine the extent of potential ocular hazard of a test substance. The tests evaluate the ocular irritation response when a test substance is administered as a single dose to the eye of a rabbit. Developed by Griffith et al. (1980), the LVET differs from the Draize rabbit eye test primarily by applying 10 μL (instead of 100 μL) of a test substance directly on the cornea (instead of the conjunctival sac) (**Table 3-1**). Scoring of corneal, iridal, and conjunctival lesions in the LVET is identical to that of the Draize rabbit eye test (**Table 3-2**).

Table 3-1 Comparison of LVET and Draize Rabbit Eye Test Protocols

	LVET	**Draize**
Dose volume	10 μL	100 μL
Dose location	Applied directly onto the cornea	Applied into the lower conjunctival sac
Eyelid closure	No forced eyelid closure	Eyelids held closed for one second
Scale for scoring ocular lesions	Draize	Draize

Abbreviation: LVET = low volume eye test

To date, the LVET has not been demonstrated as an adequately valid *in vivo* reference test method. It has not been formally adopted by any regulatory agency. For this reason, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is reviewing the validity of the LVET as an acceptable *in vivo* reference test method. In February 2007, the International Association for Soaps, Detergents and Maintenance Products (A.I.S.E.) submitted a background review document to the European Centre for the Validation of Alternative Methods (ECVAM) for an independent peer review by their Scientific Advisory Committee. The A.I.S.E. background review document provides a comprehensive summary of available data and information with which to evaluate the usefulness and limitations of the LVET.

Since its original development, proponents of the LVET have suggested that it is a more appropriate *in vivo* reference test method for comparisons to *in vitro* data than is the Draize rabbit eye test. This is based primarily on the assertion that the LVET is more representative of the human response to a potential ocular hazard than the Draize rabbit eye test, given that the site (corneal surface) and volume of exposure used in the LVET more closely resemble that of accidental human exposure. As a result, a reported advantage of the LVET is that it underpredicts the Draize test and is thereby less overpredictive of the human response than the Draize test. However, definitive data to support this claim are not available.

Table 3-2 Scale of Weighted Scores for Grading the Severity of Ocular Lesions

Cornea	
Lesion	**Score[1]**
A. Opacity – Degree of density (area which is most dense is taken for reading)	
Scattered or diffuse area – details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4
B. Area of cornea involved	
One quarter (or less) but not zero	1
Greater than one quarter but less than one half	2
Greater than one half but less than three quarters	3
Greater than three quarters up to whole area	4
Score equals A x B x 5	Total maximum = 80
Iris	
Lesion	**Score[1]**
A. Values	
Folds above normal, congestion, swelling, circumcorneal injection (any one or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage; gross destruction (any one or all of these)	2
Score equals A x 5	Total possible maximum = 10
Conjunctiva	
Lesion	**Score[1]**
A. Redness (refers to palpebral conjunctiva only)	
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3
B. Chemosis	
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of the lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4
C. Discharge	
Any amount different from normal (does not include small amount observed in inner canthus of normal animals	1
Discharge with moistening of the lids and hairs just adjacent to the lids	2
Discharge with moistening of the lids and considerable area around the eye	3
Score equals (A + B + C) x 2	Total maximum = 20

From Draize et al. (1944).

[1] The maximum total score is the sum of all scores obtained for the cornea, iris and conjunctiva. Scores of 0 are assigned for each parameter if the cornea, iris, or conjunctiva is normal.

4.0 Performance of the Low Volume Eye Test vs. the Draize Rabbit Eye Test

In general, most of the original data generated with the LVET were from surfactant-based mixtures or surfactant-based products (Freeberg et al. 1984; Gettings et al. 1996a, 1998). A comparison of the substances that have been classified by the Draize rabbit eye test as ocular corrosives or severe irritants that have also been tested in the LVET indicates that the LVET routinely underpredicts the ocular corrosive or severe irritant response in the Draize, in many cases by more than one hazard category. Gettings et al. (1996a, 1998) illustrate this in their evaluation of 25 surfactant-containing formulations and the resulting hazard classifications according to the EPA and GHS classification systems (EPA 2003; UN 2007) (**Tables 4-1** and **4-2**).

Table 4-1 Performance of the LVET in Identifying Ocular Hazard Classification According to the EPA Classification System When Compared to Draize Rabbit Eye Test Results

EPA Category[1]		LVET Classification				
		I	II	III	IV	Totals
Draize	I	3	1	6	0	10
	II	0	0	0	0	0
	III	0	0	9	2	11
	IV	0	0	0	4	4
	Totals	3	1	15	6	25

From Gettings et. al. 1996a and 1998.

Abbreviations: EPA = Environmental Protection Agency; LVET = low volume eye test

[1] EPA classification system (EPA 2003).

Table 4-2 Performance of the LVET in Identifying Ocular Hazard Classification According to the GHS Classification System When Compared to Draize Rabbit Eye Test Results

GHS Category[1]		LVET Classification				
		1	2A	2B	Not Labeled	Totals
Draize	1	0	0	4	4	8
	2A	0	0	0	0	0
	2B	0	0	0	1	1
	Not Labeled	0	0	0	16	16
	Totals	0	0	4	21	25

From Gettings et. al. 1996a and 1998.

Abbreviations: GHS = Globally Harmonized System; LVET = low volume eye test

[1] GHS classification system (UN 2007).

Tables 4-1 and **4-2** show multiple instances of underprediction of an ocular corrosive or severe irritant response in the Draize rabbit eye test by the LVET. When the EPA hazard classification system (EPA 2003) was used, the LVET underpredicted 60% (6/10) of Draize Category I substances as Category III (mild irritant) (**Table 4-3**). When the GHS hazard classification system (UN 2007) was used, the LVET underpredicted all eight of the Draize Category 1 substances: 50% (4/8) as

Category 2B (mild irritant) and 50% (4/8) as Not Labeled (not labeled as an irritant) (**Table 4-4**). These data raise concern about the ability of the LVET to reliably detect ocular corrosives or severe irritants (i.e., EPA Category I, EU Category R41, GHS Category 1).

Table 4-3 Extent of Underprediction of LVET vs. Draize Rabbit Eye Test Results According to the EPA Classification System[1]

EPA Category	LVET Category	Product
Category I	Category II	HZY (Antidandruff shampoo)
Category I	Category III	HZA (Shampoo #7)
Category I	Category III	HZE (Gel cleanser)
Category I	Category III	HZF (Baby shampoo #2)
Category I	Category III	HZL (Foam bath)
Category I	Category III	HZR (Facial cleaning foam)
Category I	Category III	HZX (Shampoo #2)

Abbreviations: EPA = Environmental Protection Agency; LVET = low volume eye test

[1] EPA classification system (EPA 2003).

Table 4-4 Extent of Underprediction of LVET vs. Draize Rabbit Eye Test Results According to the GHS Classification System[1]

GHS Category	LVET Category	Product
Category 1	Category 2B	HZI (Skin cleanser)
Category 1	Category 2B	HZK (Bubble bath)
Category 1	Category 2B	HZS (Shower gel)
Category 1	Category 2B	HZY (Antidandruff shampoo)
Category 1	Not Classified	HZL (Foam bath)
Category 1	Not Classified	HZF (Baby shampoo #2)
Category 1	Not Classified	HZX (Shampoo #2)
Category 1	Not Classified	HZA (Shampoo #7)

Abbreviations: GHS = United Nations Globally Harmonized System; LVET = low volume eye test

[1]GHS classification system (UN 2007).

Gettings et al. (1996b) published another study investigating the relationship between the LVET and Draize eye irritation test data for 10 representative hydroalcoholic personal-care formulations. **Table 4-5** provides the eye irritation profile for each of the 10 substances tested. A range of irritancy classification was demonstrated for the LVET; however, only one of the test substances was considered moderately irritating and none severely irritating according to the criteria developed by Kay and Calandra (1962). A further comparison of the LVET using the classification scheme of Bruner et al. (1992) revealed a range of responses from nonirritating to moderately irritating. The Bruner et al. (1992) LVET classification appeared to be more consistent with the Kay and Calandra irritancy classification as determined by the Draize rabbit eye test (**Table 4-5**).

Table 4-5 Summary of Available Rabbit LVET and Draize Data from Gettings et al. (1996b)

	Rabbit LVET			Rabbit Draize	
Ethanol (%)	MAS	Category[1]	Category[2]	MAS	Category[1]
5	2.2	PNI	I	7.7	Mild
10	1.3	PNI	I	3.0	Minim
15	0.7	PNI	I	0.7	PNI
20	0.7	PNI	I	0.7	PNI
33	4.3	Minim	I	14.3	Mild
40	15.5	Mild	III	38.7	Moderate
55	14.3	Mild	II	36.7	Moderate
65	22.5	Mild	III	28.3	Moderate
83	22.5	Mild	III	36.0	Moderate
90	26.0	Moderate	III	45.7	Moderate

Modified from Gettings et al. (1996b).

Abbreviations: LVET = low volume eye test; MAS = maximum average score.

[1] Kay and Calandra (1962): PNI = practically nonirritating; Minim = minimally irritating; Mild = mildly irritating; Moderate = moderately irritating.

[2] Bruner et al. (1992): I = none to inconsequential irritation (LVET-MAS = 0–5); II = slight irritation (LVET-MAS > 5–15); III = moderate to severe irritation (LVET-MAS > 15–50); IV = severe irritation (LVET-MAS > 50–65); V = extremely irritating to corrosive (LVET-MAS > 65–110).

The authors noted a similarity between the irritant responses observed in the Draize rabbit eye test and the LVET, with both tests ranking the substances in a similar order. In addition, the observed irritation for both tests significantly increased when ethanol levels exceeded 33%. Indeed, the LVET consistently underpredicted ethanol solutions above this range when compared to the Draize rabbit eye test data (**Table 4-5**).

Maurer et al. (2001a, 2001b) used pathology to evaluate the relationship of the ocular irritation response to the extent of initial injury for several nonsurfactant materials using the LVET. In these studies, they reported maximum average score (MAS) data for the LVET and irritation classifications based on Kay and Calandra (1962) as shown in **Table 4-6**. These LVET data are compared to available Draize data obtained from the database of the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC 1998) in **Table 4-7**. Maurer et al. (2001a, 2001b) applied test substances directly to the cornea and performed macroscopic assessments for irritation 3 hours after dosing and periodically thereafter up to 35 days. The alcohols, cyclohexanol and parafluoroaniline, were moderate to severe irritants in the LVET. Only cyclohexanol was tested in the Draize test, and it was a severe irritant/corrosive. Of the aldehydes, acetone was a mild irritant in the LVET and a moderate irritant in the Draize test. Formaldehyde (37%; w/v) was a severe irritant in the LVET but was not tested in the Draize test.

Four bleaches, sodium perborate monohydrate ($NaBO_3$), sodium hypochlorite (NaOCl), 10% hydrogen peroxide (H_2O_2), and 15% H_2O_2, were evaluated in the LVET, but no corresponding Draize data were available. $NaBO_3$ and NaOCl were classified as mild and minimal irritants in LVET respectively, with corneal injuries being limited to the epithelium and superficial stroma, as determined using *in vivo* confocal microscopy. It should be noted that some Material Safety Data Sheets (MSDS) from various manufacturers label NaOCl as moderately irritating or a severe irritant/corrosive in humans at or above 5.25%, while label it corrosive in humans above 14%. The

15% H_2O_2 solution would be classified as a severe irritant based on LVET data. Both concentrations affected the epithelium and deep stroma, as determined using *in vivo* confocal microscopy. In undiluted form, H_2O_2 is a known human ocular corrosive/severe irritant.

Table 4-6 Summary of MAS Categorization Data

MAS Score	Ocular Irritation Rating
0–0.5	Nonirritating— NI
0.5–2.5	Practically nonirritating— PNI
2.5–15	Minimally irritating— Minim
15–25	Mildly irritating— Mild
25–50	Moderately irritating— Moderate
50–80	Severely irritating— Severe
80–100	Extremely irritating— EI
100–110	Maximally irritating— MI

From Kay and Calandra (1962).

Abbreviation: MAS = maximum average score.

Table 4-7 Summary of Available Rabbit LVET Data

Chemical Class	Eye Data			
	Rabbit LVET		Rabbit Draize	
	MAS	Category[1]	MMAS	Category[2]
Alcohols	-	-	-	-
Cyclohexanol	50.8	Moderate/Severe	79.8	1/I/R41
Parafluoroaniline	55.0	Moderate/Severe	69.8	
Aldehydes	-	-	-	-
Acetone	19.1	Mild	65.8	2A/II/R36
Formaldehyde, 37% (w/v)	80.0	Severe		
Bleaching Agents	-	-	-	-
Sodium Perborate Monohydrate	23 ± 31.2	Mild	-	-
Sodium Hypochlorite (2.4%)	11 ± 3.6	Minim	-	-
10% Hydrogen Peroxide	16 ± 7.5	Mild	-	-
15% Hydrogen Peroxide	58.3 ± 26.1	Severe	-	-

Data from Maurer et al. (2001a, 2001b).

Abbreviations: ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; LVET = low volume eye test; MAS = maximum average score; MMAS = mean maximum average score;

[1] MAS categorization data compiled from classification table of Kay and Calandra (1962): PNI = practically nonirritating; Minim = minimally irritating; Mild = mildly irritating; Moderate = moderately irritating; Severe = severely irritating. Eye irritancy classification scores based on *in vivo* confocal microscopy and light microscopy also available in Jester (2006).

[2] Data obtained from ECETOC database (ECETOC 1998). Hazard classifications based on the Globally Harmonized System (UN 2007)/EPA (EPA 2003)/European Union (EU 2001) were determined by NICEATM based on available ECETOC Draize data.

Maurer et al. (2001a, 2001b) concluded that results obtained on these nonsurfactant materials support their hypothesis that ocular irritation is principally defined by the extent of initial injury, despite clear differences in the means by which irritants cause tissue damage.

Jester (2006) used the LVET to investigate the ocular irritancy of 22 substances varying in type (i.e., surfactant, acid, alkali, bleach, alcohol, aldehyde, and acetone) and severity (**Table 4-8**). Jester evaluated the extent of ocular irritation using light microscopy, *in vivo* confocal microscopy, and laser scanning confocal microscopy. Of the 22 substances, five produced slight irritation, nine produced mild irritation, three produced moderate/severe irritation, and five produced severe irritation. However, of the three substances for which Draize data were identified (i.e., 10% acetic acid, cyclohexanol, and acetone), the LVET underpredicted Draize results.

Table 4-8 Summary of Available Rabbit LVET Data

Chemical Class	Eye Data					
	Human[2]		Rabbit LVET		Rabbit Draize[1]	
			MAS	Category[3]	MMAS	Category[4]
Surfactant	-	-	-	-	-	-
Nonionic	-	-	-	-	-	-
Polyoxyethylene glycol monoalkylether	-	-	0.0	NI	-	-
Polyoxyethelenesorbitan	-	-	0.0	NI	-	-
Alkyl E ethoxylate	-	-	33.0	Moderate	-	-
Anionic	-	-	-	-	-	-
Sodium lauryl sulfate, 5%	-	-	4.8	Minim	-	-
Sodium linear alkylbenzene sulfonate	-	-	49.3	Moderate	-	-
Sodium alkyl ethoxylate sulfate	-	-	31.2	Moderate	-	-
Cationic	-	-	-	-	-	-
Cetyltrimethylammonium chloride, 50%	-	-	76.3	Severe	-	-
3-Isotridecyloxypropyl-bis(polyoxyethylene) ammonium chloride	-	-	7.7	Minim	-	-
3-Decyloxypropyl-bis(polyoxyethylene amine, 5%	-	-	40.0	Moderate	-	-
Alkylbenyldimethylammonium chloride, 10%		-	70.6	Severe	-	-
Acid	-	-	-	-	-	-
3% Acetic Acid	-	-	5.0	Minim	-	-
10% Acetic Acid	-	-	9.5	Minim	68	1/I/R41
Base	-	-	-	-	-	-
2% Sodium Hydroxide	-	-	5.0	Minim	-	-
8% Sodium Hydroxide	-	-	50.8	Severe	-	-

continued

Table 4-8 Summary of Available Rabbit LVET Data (continued)

Chemical Class	Eye Data					
	Human[2]		Rabbit LVET		Rabbit Draize[1]	
			MAS	Category[3]	MMAS	Category[4]
Aldehyde	-	-	-	-	-	-
Acetone	-	-	3.8	Minim	65.8	2A/II/R36
Formaldehyde, 37%	-	-	79.7	Severe	-	-
Alcohol	-	-	-	-	-	-
Parafluoroaniline	-	-	43.3	Moderate	-	-
Cyclohexanol	-	-	45.8	Moderate	79.8	1/I/R41
Bleach	-	-	-	-	-	-
Sodium Perborate Monohydrate	-	-	8.3	Minim	-	-
Sodium Hypochlorite (2.4%)	Severe[5]	-	11.8	Minim	-	-
10% Hydrogen Peroxide	-	-	30.3	Moderate	-	-
15% Hydrogen Peroxide	-	-	68.3	Severe	-	-

Data from Jester (2006).

Abbreviations: ATSDR = Agency for Toxic Substances and Disease Registry; ECETOC = European Centre for Ecotoxicology and Toxicology of Chemicals; LVET = low volume eye test; MAS = maximum average score; MMAS = mean maximum average score; MSDS = material safety data sheet.

[1] Data obtained from ECETOC database (ECETOC 1998). Hazard classifications based on EPA (EPA 2003), Globally Harmonized System (UN 2007), and European Union (EU 2001) were determined by the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods based on ECETOC Draize data.

[2] Data compiled from accidental exposures (ATSDR database).

[3] MAS categorization data compiled from classification table of Kay and Calandra, (1962) (see Table 4-8). Eye irritancy classification scores based on *in vivo* confocal microscopy and light microscopy also available in Jester (2006).

[4] Category classification– EPA/GHS/EU.

[5] Labeled as moderately irritating or severe irritant/corrosive in humans at or above 5.25% based on some MSDS reports, while labeled as corrosive in humans above 14%.

5.0 Performance of the Low Volume Eye Test vs. the Draize Rabbit Eye Test Considering Human Study Data and Experience

Human data on potential ocular hazards are available either from accidental exposures or from clinical studies. Accidental exposures are not generally considered to be a reliable source of the true ocular hazard potential because such exposures are likely immediately followed by flushing the eyes with large volumes of water. Thus they may not represent the most severe lesion that might be produced by such an exposure. Griffith et al. (1980) conducted a series of rabbit eye test studies using either 10 or 100 μL of substances "recognized as slightly irritating, moderately irritating, or severely irritating/corrosive to humans."

The ocular corrosive or severe irritant substances included the following:

- Acetic acid (10%), which is referenced as a severe irritant based on splashes of vinegar (containing 4% to 10% acetic acid) reported to cause pain, conjunctival hyperemia, and occasionally permanent opacity of the human cornea
- Calcium hydroxide (hydrated lime), which is referenced as one of the most common causes of severe chemical burns of the eye (McLaughlin 1946; Grant and Schuman 1993)
- Formaldehyde (38%), which is referenced for the range of injuries caused by splashes in the human eye from minor transient discomfort to severe, permanent corneal opacities (Grant and Schuman 1993)

Although detailed animal data are not available, the summary data provided by Griffith et al. (1980) indicate that the lesions induced by either 10 or 100 μL of these substances were not reversible within 21 days. However, such accidental exposures as human reference data make definitive quantitative measures of amount and time of exposure impossible to obtain. Ethical considerations and results based largely on the Draize rabbit eye test have limited the severity of substances that can be tested in human clinical studies. As a result, comparisons to human data are based on clinical study tests with mild irritants or substances not labeled as irritants. Such data provide little assurance to the regulatory agencies charged with protecting public health that the LVET can provide adequate protection from substances that may cause moderate or severe ocular injuries.

The fact that seemingly innocuous commercial consumer products were identified as ocular corrosives or severe irritants by the Draize eye test could be seen as supporting the contention that the Draize eye test is excessively overpredictive of the actual hazard to humans. However, because of the paucity of information on the performance of known human corrosives in the LVET, these data cannot simply be dismissed.

Several studies have published supporting data for the demonstrated usefulness of the LVET (Ghassemi et al. 1993; Roggeband et al. 2000; Freeberg et al. 1984, 1986a, 1986b).

5.1 Ghassemi et al. (1993)

Ghassemi et al. (1993) provides an evaluation of *a single product*, a liquid household cleaner (pH 3) reportedly containing the following qualitative formula: nonionic surfactant, amphoteric surfactant, hydrotrope, solvent, and water. This study directly compares LVET results to human clinical data (using either 10 or 100 μL doses) for the same test substance. No Draize rabbit eye test data had been reported; therefore, LVET results could not be compared to those of the standard eye test. The ocular lesions that were produced in this study and their subsequent time to clear suggest that this product is a mild ocular irritant (**Table 5-1**). The authors conclude that because the direct application to the human eye using either 10 or 100 μL doses produced similar results, the smaller volume for testing is more appropriate anatomically and physiologically based on eye volume capacity and subsequent tear volume.

Table 5-1 Summary of Rabbit and Human Responses to an Undiluted Liquid Household Cleaner

Species	Ocular Tissues Involved	Number of Eyes Affected			Mean CR at 24 hr	Eyes Cleared/ Time to Clear	Max Time to Clear
		Cornea	Iris	Conj			
Rabbit LVET	Cornea Iris Conj	3/3	2/3	3/3	2	2/4 days 1/7 days	7 days
Human (10 μL)	Conj	0/10	0/10	10/10	0.1	1/1hr; 4/2hr; 6/4hr; 10/24hr	48 hr
Human (100 μL)	Conj	0/10	0/10	10/10	0.2	1/1hr; 2/2hr; 9/24hr; 2/46hr	70hr

Data from Ghassemi et al. (1993).

Abbreviations: Conj = conjunctiva; CR = conjunctival redness; hr = hour; LVET = low volume eye test (10 μL dose volume).

5.2 Roggeband et al. (2000)

Roggeband et al. (2000) evaluates two products, a dishwashing liquid (pH 8, contains anionic surfactant, nonionic surfactant, soap, ethanol, water) and a liquid laundry detergent (pH 7, contains anionic surfactant, nonionic surfactant, ethanol, water). This study directly compares modified LVET results to those of a human clinical study. Both rabbits and humans were dosed with either 3 μL (dishwashing detergent) or 1 μL (liquid laundry detergent) of the test products. There are no corresponding Draize rabbit eye test data. The ocular lesions that were produced in this study and their subsequent time to clear suggest that these products are mild ocular irritants (**Table 5-2**). The authors conclude that these data support the notion that (1) an accidental exposure would be approximately 10 μL or less and (2) a volume of 10 μL provides a suitable margin of safety. This is based on (1) knowledge of the anatomical and physiological characteristics of the eye and (2) the fact that study participants in Roggeband et al. (2000) could "only be exposed to 1 μL of dishwashing liquid and 3 μL of liquid laundry detergent before predetermined 'cut-off' ocular responses were observed above which it would have been ethically unacceptable to proceed" (Roggeband et al. 2000).

Table 5-2 Human and Rabbit Eye Responses to a Liquid Laundry Detergent (1 µL)

Human Volunteer	Human				Animal Number	Rabbit LVET[1]			
	1 hour		24 hours			1 hour		24 hours	
	Cornea	Conj	Cornea	Conj		Cornea	Conj	Cornea	Conj
5	0	1/1	0	0/0	28 (c)	0/0	1/1/0	1/2	2/1/1
6	0	1/0	0	0/0	29 (c)	0/0	1/1/0	1/2	2/1/1
21	0	1/0	0	0/0	30 (c)	0/0	1/1/0	0/0	2/1/1
23	1/2	1/0	0	1/0	31 (scs)	0/0	1/1/0	1/4	2/1/0
25	1/1	1/0	0	0/0	32 (scs)	0/0	1/1/0	1/3	2/1/1
27	0	1/0	0	1/0	33 (scs)	0/0	1/1/0	1/4	2/1/1
28	0	1/0	0	0/0					
30	0	0/0	0	0/0					
32	0	1/0	0	0/0					
34	0	1/0	0	0/0					

Data from Roggeband et al. (2000).

Abbreviations: (c) = test substance dosed on the central cornea; Conj = conjunctiva; LVET = low volume eye test; (scs) = test substance dosed on the superior conjunctival sac.

[1]Low volume eye test was modified to use 1 µL instead of 10 µL.

5.3 Freeberg et al. (1984)

A series of studies by Freeberg et al. (1984) compare data from LVET, Draize rabbit eye test, and human studies or experience. Freeberg et al. (1984) compares LVET and Draize rabbit eye test data for 29 cleaning products (laundry products, household cleaning products, and dishwashing products) to human experience data. The ocular lesions that were produced in this study and their subsequent time to clear suggest that these products are either mild ocular irritants or substances not labeled as irritants (**Table 5-3**). The human data were obtained from medical records of factory and consumer accidental eye exposures (515 reports over a 2-year period). The results indicate that both rabbit LVET and Draize eye tests overpredicted (based on time to clear of ocular lesions) the human response based on accidental eye exposure to the cleaning products. The time to clear was longer in the Draize eye test than in the LVET for the same product, forming the basis for the conclusion that the LVET more closely predicts the human response.

Table 5-3 Summary of Rabbit and Human Accidental Exposure Data from Freeberg et al. (1984)

Species	Test Method	Number of Products	Average ± SD Mean Time to Clear (Day Range)	Average ± SD Median Time to Clear) (Day Range)	Average ± SD Number of Incidents (Range)
Rabbit	LVET	17	7.3 ± 7.2 (1.3–28.8)	6.2 ± 8.8 (0.7–35)	Not Applicable
Rabbit	Draize	26	20.4 ± 7.2 (3.1–33.5)	20.2 ± 12.3 (1.4–35)	Not Applicable
Human	Experience data[1]	29	2.4 ± 2.1 (0.2–9.5)	1.5 ± 1.5 (0.1–1.8)	16.2 ± 8.4 (3–68)

Data from Freeberg et al. (1984).

Abbreviations: LVET = low volume eye test; SD = standard deviation.

[1]Experience data = combined manufacturing and consumer accidental exposures.

5.4 Freeberg et al. (1986a)

Freeberg et al. (1986a) compared rabbit eye test results (both LVET and Draize) with those of human studies (both 10 μL and 100 μL dose volumes) for four cleaning products (a liquid fabric softener, liquid shampoo, liquid hand soap, and liquid laundry detergent). The results indicate that the LVET overpredicted the human response to 10 μL and 100 μL of the same product. The ocular lesions in the Draize rabbit eye test (100 μL) were more severe (both type and longevity) than in the human test using the same volume. While the majority of effects in humans were conjunctival, the corneal effects in humans were minimal and transient. The corneal effects in rabbits were more severe and recovered less quickly. The ocular lesions that were produced in this study and their subsequent time to clear suggest that these products would be classified as mild ocular irritants based on the Draize rabbit eye test results, the LVET, and human results (**Table 5-4**).

Table 5-4 Human Clinical Study and Rabbit Data

Test Product	Concentration (% in water)	Time to Clear (hr)			
		Dosing Volume			
		Rabbit	Human		Rabbit
		10 μL	10 μL	100 μL	100 μL
Liquid fabric Softener	60	45	18.9	24.9	45
	80	66	12.6	33.6	93
	100	27	13.2	12.5	84
Liquid shampoo	4	5	1.5	2.5	NT
	16	19.8	1.9	2.6	36.5
	20	33	7.5	7.9	63
Liquid hand soap	8	24	1.5	31.5	63
	10	42	10.5	9.1	66
	12	42	1.7	NT	NT
Liquid laundry detergent	2	8.8	2	24.1	27.8
	3	19.8	4.7	1.8	60
	4	39.8	4.8	19.8	75

Data from Freeberg et al. (1986a).
Abbreviation: NT = not tested.

5.5 Freeberg et al. (1986b)

Freeberg et al. (1986b) compares LVET and Draize rabbit eye test data for 14 cleaning products (liquid and solid laundry products, liquid and solid household cleaning products, liquid and solid dishwashing products, and liquid shampoos) to human experience data. The ocular lesions that were produced in this study and their subsequent time to clear suggest that these products would be classified as moderate to severe ocular irritants based on the Draize rabbit eye test results. Most would be classified as mild ocular irritants by the LVET (**Table 5-5**). The human data were obtained from medical records of factory and consumer accidental eye exposures (218 reports over an 18-month period). Similar to Freeberg et al. (1986a), rabbit LVET and Draize tests both overpredicted the human response due to accidental eye exposure (based on time to clear). Because the time to clear was longer for substances tested in the Draize rabbit eye test than in the LVET, the authors concluded

that the LVET outcome more closely relates to the human experience than the Draize rabbit eye test does.

Table 5-5 Human Accidental Exposure and Rabbit Data

Product	Mean Time to Clear (Days)		
	Human	Rabbit LVET	Rabbit Draize
Liquid Laundry Product #1	1.92	26.6	35.0
Liquid Dishwashing Product #1	0.77	8.2	25.7
Solid Dishwashing Product #1	0.59	4.6	18.3
Liquid Dishwashing Product #2	0.43	7.7	11.7
Liquid Household Cleaning Product #1	0.38	-	11.1
Liquid Dishwashing Product #3	0.30	3.9	22.2
Liquid Household Cleaning Product #2	0.23	4.0	15.2
Solid Household Cleaning Product #1	0.19	1.3	29.2
Solid Dishwashing Product #1	0.08	2.1	13.8
Solid Dishwashing Product #1	0.06	2.9	15.1

Data from Freeberg et al. (1986b).

Abbreviation: LVET = low volume eye test.

6.0 Summary

Because studies conducted with the LVET have been limited to tests of surfactant-containing personal and household cleaning products, the applicability domain for which the LVET can be considered is necessarily restricted to these product types. As summarized in **Table 6-1**, LVET data have previously been used by one personal-care product company to support submission of data to the EPA for the registration of at least five antimicrobial cleaning products. The results were used by EPA reviewers in a weight-of-evidence approach, in conjunction with either consumer incidence data (i.e., commercial products for which there is an opportunity for adverse events to be reported by the consumer) and/or Draize data for similar, structurally related substances. Each study was considered on a case-by-case basis and several submissions were deemed unacceptable by the EPA because either the LVET study was not considered an acceptable fulfillment of the eye irritation data requirement and/or the further confirmatory information provided by the submitter was insufficient (**Table 6-1**). Based on the data provided to NICEATM in the Data Evaluation Reports (DERs), it appears that a final EPA ocular hazard classification was not assigned for any product using LVET data alone.

As indicated in the studies summarized above, human data on potential ocular hazards are available either from accidental exposures or from clinical studies. Accidental exposures are not generally considered to be a reliable source of the true ocular hazard potential because such exposures are likely immediately followed by flushing the eyes with large volumes of water. Such accidents make definitive quantitative measures of amount and time of exposure impossible to obtain. Although the Draize eye test is reported to be excessively overpredictive of the human response, ethical considerations based largely on results from the Draize rabbit eye test are used to limit the types of substances that can be tested in human clinical studies. As a result, comparisons to human clinical study data are based on tests of mild irritants or substances not labeled as irritants. Such data provide little assurance to the regulatory agencies charged with protecting public health that the LVET can provide adequate protection from more severe ocular injuries.

Thus, while the LVET is proposed as more likely to approximate the volume of a substance that could enter the human eye accidently, there are limited data to indicate whether it can accurately identify the ocular hazard of substances known to cause moderate, severe, or permanent human ocular injuries. In contrast, there are no documented instances in which a substance with a hazard category determined in the Draize eye test produced a more severe hazard category response in humans following accidental exposures or ethical human studies.

Table 6-1 Summary of Ocular Hazard Classifications for EPA Registered Antimicrobial Cleaning Products: Consideration of LVET Data and EPA Determinations[1]

EPA Registration Number or Submission Code	Submission Date	Animal Data from LVET Study	EPA Hazard Category Based on LVET Data	Additional Submission Information	Final EPA Classification Provided in DER
3573-AO	Jul 20, 2000	No corneal opacity, iritis, or conjunctival irritation (n=6).	Category IV	Consumer incidence data	Study unacceptable[2]
	Jun 6, 2001	Same as for Jul 20, 2000	Consumer incidence data; LVET and Draize data for similar substances		Category III

continued

Table 6-1 Summary of Ocular Hazard Classifications for EPA Registered Antimicrobial Cleaning Products: Consideration of LVET Data and EPA Determinations[1] (continued)

EPA Registration Number or Submission Code	Submission Date	Animal Data from LVET Study	EPA Hazard Category Based on LVET Data	Additional Submission Information	Final EPA Classification Provided in DER
3573-TE	Aug 9, 2000	No corneal opacity, iritis, redness, or chemosis at day 1 (n=3).	Category IV	None	Study unacceptable[2]
	Feb 7, 2001	Repeat submission from Aug 9, 2000		Animal data for similar substances	Category IV
3573-72	Jun 6, 2001	NP	Category III	Consumer incidence data; LVET and Draize data for similar substances	Category III
3573-AI	Jun 6, 2001	NP	NP	NP	Category II
S596273	Jun 27, 2001	No corneal effects or iritis observed. Conjunctivitis resolved by 72 hr (n=3).	Category III	None	Study unacceptable[2]
3573-TG	Jul 25, 2001	NP	Category III	Consumer incidence data; Animal skin irritation study-Category I (severe irritant)	Study unacceptable[3]

Abbreviations: DER = Data Evaluation Reports; EPA = U.S. Environmental Protection Agency; LVET = low volume eye test; NP = not provided (i.e., information not contained in and/or not provided to NICEATM in DERs).

[1] Data source: Obtained from a Freedom of Information Act request submitted to EPA for LVET data used to support the submission of data for the registration of antimicrobial cleaning products.

[2] "The EPA does not consider the LVET study to be an acceptable fulfillment of the eye irritation data requirement."

[3] "It is now the Product Safety Branch's (PSB) policy to take a weight of the evidence approach to the situation by considering individual LVET studies for possible acceptance on a case by case basis if they are significantly supplemented by further, confirmatory information. In the present case, that confirmatory further information is not sufficient."

7.0 References

Bruner LH, Parker RD, Bruce RD. 1992. Reducing the number of rabbits in the low-volume eye test. Fund Appl Toxicol 19:330-335.

Chiou GCY. 1999. Ophthalmic Toxicology. In: Target Organ Toxicology Series (Hayes AW, Thoams JA, Gardner DG, eds). Philadelphia, PA:CRC Press.

Clean Air Act Amendments of 1990. 1990. Public Law 101-549. Available: http://www.ehso.com/caa.htm.

CPSC. 1995. Test for eye irritants. 16 CFR 1500.42.

Draize J, Woodard G, Calvery H. 1944. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharm Exp Ther 82:377–390.

ECETOC. 1998. Eye Irritation Reference Chemicals Data Bank. 2d ed. Technical Report No. 48 (2). European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). Brussels, Belgium:ECETOC.

EPA. 1998. Health Effects Test Guideline. OPPTS 870.2400 Acute Eye Irritation. EPA 712-C-98-195. Washington, DC:U.S. Environmental Protection Agency.

EPA. 2003. Label Review Manual. 3rd ed. EPA735-B-03-001. Office of Prevention, Pesticides and Toxic Substances. Washington, DC:U.S. Environmental Protection Agency.

EU. 2001. Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities L255:1–333.

Federal Environmental Pesticide Control Act. 1972. Public Law 92-516.

Federal Hazardous Substances Act. 1964. Public Law 86-613.

Federal Insecticide, Fungicide and Rodenticide Act. 1947. Public Law 80-102.

Food, Drug and Cosmetic Act of 1938. 1938. Public Law 75-717.

Freeberg FE, Griffith JF, Bruce RD, Bay PHS. 1984. Correlation of animal test methods with human experience for household products. J Toxic Cut & Ocular Toxic 1:53–64.

Freeberg FE, Hooker DT, Griffith JF. 1986b. Correlation of animal test methods with human experience for household products: an update. J Toxic Cut & Ocular Toxic 5:115–123.

Freeberg FE, Nixon GA, Reer PJ, Weaver JE, Bruce RD, Griffith JF, et al. 1986a. Human and rabbit eye responses to chemical insult. Fundam Appl Toxicol 7:626–634.

Gettings S, Lordo R, Hintze K, Bagley D, Casterton P, Chudkowski M, et al. 1996a. The CTFA evaluation of alternatives program: an evaluation of *in vitro* alternatives to the Draize primary eye irritation test. (Phase III) surfactant-based formulations. Food Chem Toxic 34:79–117.

Gettings SD, Lordo RA, Demetrulias J, Feder PI, Hintze KL. 1996b. Comparison of low-volume, Draize and *in vitro* eye irritation test data. I. Hydroalcoholic formulations. Food Chem Toxicol 34:737–749.

Gettings SD, Lordo RA, Feder PI, Hintze KL. 1998. A comparison of low volume, Draize and *in vitro* eye irritation test data. III. Surfactant-based formulations. Food Chem Toxicol 36:209–231.

Ghassemi A, Sauers LH, Bruner LH, Reer PJ, Hall RH. 1993. Demonstrating the human safety of a new household cleaning (HSC) product using alternatives to the Draize eye irritation test. Presentation made at the U.S. Society of Toxicology New Orleans, Louisiana.

Grant WM, Schuman JS. 1993. Toxicology of the Eye: Effects on the Eyes and Visual System from Chemicals, Drugs, Metals and Minerals, Plants, Toxins, and Venoms; Also Systemic Side Effects from Eye Medications. 4th ed. Springfield, IL:Charles C. Thomas.

Griffith J, Nixon G, Bruce R, Reer P, Bannan E. 1980. Dose-response studies with chemical irritants in the albino rabbit eye as a basis for selecting optimum testing conditions for predicting hazard to the human eye. Toxic Appl Pharmacol 55:501–513.

ICCVAM. 2003. ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods. NIH Publication No. 03-4508. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

ICCVAM. 2010. ICCVAM Test Method Evaluation Report: Current Validation Status of a Proposed *In Vitro* Testing Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products. NIH Publication No. 10-7513. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

Jester JV. 2006. Extent of corneal injury as biomarker for hazard assessment and the development of alternative models to the Draize rabbit eye test. Cutan Ocul Toxicol 25:41–54.

Kay JH, Calandra JC. 1962. Interpretation of eye irritation tests. J Soc Cosmet Chem 13:281–289.

Maurer JK, Molai A, Parker RD, Li L, Carr GJ, Petroll WM, Cavanagh HD, Jester JV. 2001a. Pathology of ocular irritation with bleaching agents in the rabbit low-volume eye test Toxicologic Pathology 29:308–319.

Maurer JK, Molai A, Parker RD, Li L, Carr GJ, Petroll WM, Cavanagh HD, Jester JV. 2001b. Pathology of ocular irritation with acetone, cyclohexanol, parafluoroaniline, and formaldehyde in the rabbit low-volume eye test. Toxicologic Pathology 29:187–199.

McGwin G. Hall TA, Xie A, Owsley C. 2006. Trends in eye injury in the United States, 1992-2001. Inv Ophthalmol Vis Science 47:521–527.

McLaughlin R. 1946. Chemical burns of the human cornea. Am J Ophthamol 29:1355–1362.

Occupational Safety and Health Act of 1970. 1970. Public Law 91-596

Organisation for Economic Co-operation and Development. 2002. Test guideline 405, Acute eye irritation/corrosion, adopted February 24, 1987. In: OECD Guidelines for Testing of Chemicals. Paris:OECD.

Roggeband R, York M, Pericoi M, Braun W. 2000. Eye irritation responses in rabbit and man after single applications of equal volumes of undiluted model liquid detergent products. Food Chem Toxic 38:727–734.

Toxic Substances Control Act of 1976. 1976. Public Law 94-469.

United Nations. 2007. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). New York, Geneva:United Nations Publications.

Wagoner MD. 1997. Chemical injuries of the eye: Current concepts in pathophysiology and therapy. Surv Ophthalmol 41:275–313.

Wilhelmus K. 2001. The Draize eye test. Survey of Ophthalmology 45:493–515.

8.0 Glossary[1]

Assay:[2] The experimental system used. Often used interchangeably with *test* and *test method.*

Canthus: The angle formed by the meeting of the upper and lower eyelids at either side of the eye.

Chemosis: A form of eye irritation in which the membranes that line the eyelids and surface of the eye (*conjunctiva*) become swollen.

Classification system: An arrangement of quantified results or data into groups or categories according to previously established criteria.

Confocal microscopy: An optical imaging technique that increases the contrast of micrographs. It can used to reconstruct three-dimensional images by use of a spatial pinhole to eliminate out-of-focus light or flare in specimens that are thicker than the focal plane.

Conjunctiva: The mucous membrane that lines the inner surfaces of the eyelids and folds back to cover the front surface of the eyeball, except for the central clear portion of the outer eye (the cornea). The conjunctiva is composed of three sections: palpebral conjunctiva, bulbar conjunctiva, and fornix.

Conjunctival sac: The space located between the eyelid and the conjunctiva-covered eyeball. Substances are instilled into the sac to conduct an *in vivo* eye test.

Cornea: The transparent part of the coat of the eyeball that covers the iris and pupil and admits light to the interior.

Corneal opacity: A subjective measurement of the extent of opaqueness of the cornea following exposure to a test substance. Increased corneal opacity is indicative of damage to the cornea.

Corneal stroma: The substantia propia: a tough, fibrous, transparent layer consisting of plates of collagen fibrils (lamellae) produced by keratocytes that make up 10% of the stroma. The fibrils run parallel to each other, but are positioned at right angles to adjacent lamellae.

Corrosion: Destruction of tissue at the site of contact with a substance.

Corrosive: A substance that causes irreversible tissue damage at the site of contact.

Distress: To cause pain, or stress, or suffering to.

Endpoint:[2] The biological process, response, or effect assessed by a test method.

Globally Harmonized System (GHS): A classification system presented by the United Nations that provides (a) a harmonized criteria for classifying substances and mixtures according to their health, environmental and physical hazards, and (b) harmonized hazard communication elements, including requirements for labeling and safety data sheets.

Hazard:[2] The potential for an adverse health or ecological effect. A hazard potential results only if an exposure occurs that leads to the possibility of an adverse effect being manifested.

Hyperemia: An increase in blood flow to a tissue (e.g., cornea).

In vitro: In glass. Refers to assays that are carried out in an artificial system (e.g., in a test tube or petri dish) and typically use single-cell organisms, cultured cells, cell-free extracts, or purified cellular components.

---

[1] The definitions in this Glossary are restricted to their uses with respect to the Draize rabbit eye test method and in the assessment or treatment of pain and distress.

[2] Definition used by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM 2003)

In vivo: In the living organism. Refers to assays performed in multicellular organisms.

Iris: The contractile diaphragm perforated by the pupil and forming the colored portion of the eye.

Not Labeled: (a) A substance the produces no changes in the eye following application to the anterior surface of the eye. (b) Substances that are not classified as GHS Category 1, 2A, or 2B; or EU R41 or R36 ocular irritants.

Ocular: Of or relating to the eye.

Ocular corrosive: A substance that causes irreversible tissue damage in the eye following application to the anterior surface of the eye.

Ocular irritant: A substance that produces a reversible change in the eye following application to the anterior surface of the eye.

Pain: An unpleasant sensation occurring in varying degrees of severity as a consequence of injury, disease, or emotional disorder; suffering or distress.

pH: A measure of the acidity or alkalinity of a solution. A pH of 7.0 is neutral; higher pHs are alkaline, lower pHs are acidic.

Protocol:[2] The precise, step-by-step description of a test, including the listing of all necessary reagents, criteria and procedures for the evaluation of the test data.

Severe irritant: (a) A substance that causes tissue damage in the eye following application to the anterior surface of the eye that is not reversible within 21 days of application or causes serious physical decay of vision. (b) Substances that are classified as GHS Category 1, EPA Category I, or EU R41 ocular irritants.

Test:[2] The experimental system used; used interchangeably with *test method* and *assay*.

Test method:[2] A process or procedure used to obtain information on the characteristics of a substance or agent. Toxicological test methods generate information regarding the ability of a substance or agent to produce a specified biological effect under specified conditions. Used interchangeably with *test* and *assay*. See also *validated test method* and *reference test*.

Validated test method:[2] An accepted test method for which validation studies have been completed to determine the relevance and reliability of this method for a specific proposed use.

Validation:[2] The process by which the reliability and relevance of a procedure are established for a specific purpose.

Weight of evidence (process): The strengths and weaknesses of a collection of information are used as the basis for a conclusion that may not be evident from the individual data.

Appendix C

Independent Scientific Peer Review Panel Assessment

This page intentionally left blank

Appendix C1

Summary Minutes from the Peer Review Panel Meeting on May 19-21, 2009

This page intentionally left blank

Summary Minutes

Independent Scientific Peer Review Panel Meeting

Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches

Consumer Product Safety Commission Headquarters
Fourth Floor Hearing Room
Bethesda Towers Building
Bethesda, MD

May 19 - 21, 2009

Peer Review Panel Members:

A. Wallace Hayes, Ph.D., DABT, FATS, ERT (Peer Review Panel Chair)	Visiting Scientist (Harvard), Harvard School of Public Health, Andover, MA; Principal Advisor, Spherix Incorporated, Bethesda, MD
Hongshik Ahn, Ph.D.	Professor, Stony Brook University, Stony Brook, NY
Paul Bailey, Ph.D.	Bailey & Associates Consulting, Neshanic Station, NJ
Richard Dubielzig, D.V.M.	Professor, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI
Henry Edelhauser, Ph.D.[1]	Professor of Ophthalmology and Director of Ophthalmic Research, Emory University School of Medicine, Atlanta, GA
Mark Evans, D.V.M., Ph.D., DACVP	Pathology Lead for Ophthalmology Therapeutic Area, Pfizer Global Research and Development at La Jolla Drug Safety Research and Development, San Diego, CA
James Jester, Ph.D.	Professor of Ophthalmology and Biomedical Engineering, Endowed Chair, University of California-Irving, Orange, CA

[1] Unable to attend the Panel meeting, but participated in the review of all materials.

Peer Review Panel Members:

Tadashi Kosaka, D.V.M., Ph.D.	Associate Director, Chief, Laboratory of Immunotoxicology and Acute Toxicology, Toxicology Division, The Institute of Environmental Toxicology, Ibaraki, Japan
Alison McLaughlin, M.Sc., DABT	Health Canada, Environmental Impact Initiative, Office of Science and Risk Management, Health Products and Food Branch, Ottawa, Ontario, Canada
J. Lynn Palmer, Ph.D.	Associate Professor, Department of Palliative Care and Rehabilitation Medicine, University of Texas, MD Anderson Cancer Center, Houston, TX
Robert Peiffer, Jr., D.V.M., Ph.D., DACVO	Senior Investigator, Merck Research Laboratories, Safety Assessment Toxicology, West Point, PA
Denise Rodeheaver, Ph.D., DABT	Assistant Director, Alcon Research Ltd., Department of Toxicology, Fort Worth, TX
Donald Sawyer, D.V.M., Ph.D., DACVA	Professor Emeritus, Retired, College of Veterinary Medicine, Michigan State University, East Lansing, MI
Kirk Tarlo, Ph.D., DABT	Scientific Director, Comparative Biology and Safety Sciences, Amgen, Inc., Thousand Oaks, CA
Daryl Thake, D.V.M., Dipl. ACVP[1]	Midwest ToxPath Sciences, Inc., Chesterfield, MO
Scheffer Tseng, M.D., Ph.D.[1]	Director, Ocular Surface (OS) Center, Medical Director OS Research & Education Foundation, Directory R&D Department, Tissue Tech, Inc., Ocular Surface Center, P.A., Miami, FL
Jan van der Valk, Ph.D.	Senior Scientist, Departments of Animals, Science and Society, Faculty of Veterinary Medicine, Utrecht University, Netherlands Centre Alternatives to Animal Use (NCA), Utrecht, Netherlands
Philippe Vanparys, Ph.D., DABT	Managing Director, CARDAM (VITO), Mol, Belgium
Maria Pilar Vinardell, Ph.D.	Director, Department of Physiology, Professor of Physiology and Pathology, Department Fisologia, Facultat de Farmacia, Universitat de Barcelona, Barcelona, Spain
Sherry Ward, Ph.D., M.B.A.	In Vitro Toxicology Consultant, BioTred Solutions, Science Advisor, International Foundation for Ethical Research, New Market, MD

Peer Review Panel Members:

Daniel Wilson, Ph.D., DABT	Mammalian Toxicology Consultant, Toxicology and Environmental Research Consulting, The Dow Chemical Company, Midland, MI
Fu-Shin Yu, Ph.D.	Director of Research, Department of Ophthalmology & Anatomy, School of Medicine, Wayne State University, Detroit, MI

ICCVAM and ICCVAM Ocular Toxicity Working Group Members:

Meta Bonner, Ph.D.	EPA, OPP, Washington, DC
Robert Bronaugh, Ph.D.	FDA, CFSAN, College Park, MD
Pertti Hakkinen	NLM, Bethesda, MD
Masih Hashim, D.V.M., Ph.D.	EPA, OPP, Washington, DC
Jodie Kulpa-Eddy, D.V.M. (ICCVAM Vice-Chair)	USDA, Riverdale, MD
Donnie Lowther	FDA, CFSAN, College Park, MD
Deborah McCall	EPA, OPP, Washington, DC
Jill Merrill, Ph.D. (OTWG Chair)	FDA, CDER, Silver Spring, MD
John Redden	EPA, OPP, Crystal City, VA
RADM William Stokes, D.V.M., DACLAM (Director, NICEATM)	NIEHS, Research Triangle Park, NC
Marilyn Wind, Ph.D., (ICCVAM Chair)	CPSC, Bethesda, MD

Invited Experts:

Rodger Curren, Ph.D.	Institute for In Vitro Sciences (IIVS), Gaithersburg, MD
Arnhild Schrage, Ph.D.	Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany

European Centre for the Validation of Alternative Methods, ICCVAM OTWG Liaison:

João Barroso, Ph.D.	European Centre for the Validation of Alternative Methods, Ispra, Italy

Public Attendees:

Attendee	**Affiliation**	**Day Attended**		
		1	**2**	**3**
Odelle Alexander	Syngenta Crop Protection, Greensboro, NC	√	√	√
Ian Blackwell	EPA, Antimicrobials Division, Arlington, VA	√	√	-
Krishna Deb	EPA, Antimicrobials Division, Arlington, VA	√	√	-
Noe Galvan	Clorox Services Co., Pleasanton, CA	√	√	√
Earl Goad	EPA, Antimicrobials Division, Arlington, VA	√	√	√
John Harbell	Mary Kay Inc., Addison, TX	√	√	√
Leon Johnson	EPA, Antimicrobials Division, Crystal City, VA	√	-	-
Eli Kumekpor	Invitrogen, Frederick, MD	√	-	√
Pauline McNamee	The Procter & Gamble Co., Egham, Surrey, U.K.	√	√	√
Michelle Piehl	MB Research Laboratories, Spinnerstown, PA	√	-	-
Patrick Quinn	Accord Group, Washington, DC	-	-	√
Hans Raabe	Institute for In Vitro Sciences, Gaithersburg, MD	-	√	√
Mary Richardson	Bausch & Lomb, Rochester, NY	√	√	√
Michael Rohovsky	Johnson & Johnson, New Brunswick, NJ	√	√	√
Kristie Sullivan	Physicians Committee for Responsible Medicine, Oakland, CA	-	-	√
Neil Wilcox	Consultant/FDA, College Park, MD	√	√	-

NICEATM:

RADM William Stokes, D.V.M., DACLAM	Director
Debbie McCarley	Special Assistant to the Director

Support Contract Staff— Integrated Laboratory Systems, Inc.:

David Allen, Ph.D.	Elizabeth Lipscomb, Ph.D.
Jonathan Hamm, Ph.D.	Linda Litchfield
Nelson Johnson	Greg Moyer, M.B.A.
Brett Jones, Ph.D.	James Truax, M.A.

Abbreviations used in participants' affiliations:

CDER = Center for Drug Evaluation and Research

CFSAN = Center for Food Safety and Applied Nutrition

CPSC = U.S. Consumer Product Safety Commission

ECVAM = European Centre for the Validation of Alternative Methods

EPA = U.S. Environmental Protection Agency

FDA = U.S. Food and Drug Administration

ICCVAM = Interagency Coordinating Committee on the Validation of Alternative Methods

ILS = Integrated Laboratory Systems, Inc.

NICEATM = National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

NIEHS = National Institute of Environmental Health Sciences

NLM = National Library of Medicine

OPP = Office of Pesticide Products

OTWG = Ocular Toxicity Working Group

USDA = U.S. Department of Agriculture

TUESDAY, MAY 19, 2009

Call to Order and Introductions

Dr. Hayes (Peer Review Panel Chair) called the meeting to order at 8:30 a.m. and introduced himself. He then asked all Peer Review Panel (Panel) members to introduce themselves and to state their name and affiliation for the record. He then asked all the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) staff, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) members, the ICCVAM Ocular Toxicity Working Group (OTWG) members, the European Centre for the Validation of Alternative Methods (ECVAM) staff person, and members of the public to introduce themselves. Dr. Hayes stated that there would be opportunities for public comments during the discussions associated with each of the ten test method topics. He asked that those individuals interested in making a comment register at the registration table and provide a written copy of their comments, if available, to NICEATM staff. Dr. Hayes emphasized that the comments would be limited to seven minutes per individual per public comment session, and that, while an individual would be welcome to make comments during each commenting period, repeating the same comments at each comment period would be inappropriate. He further stated that the meeting was being recorded and that Panel members should speak directly into the microphone.

Welcome from the ICCVAM Chair

Dr. Wind, U.S. Consumer Product Safety Commission (CPSC) and Chair of ICCVAM, welcomed everyone to CPSC and to the Panel meeting. Dr. Wind stressed the importance of this Panel's efforts, especially considering the public health importance of ocular safety testing and hazard labeling. Dr. Wind noted that approximately 125,000 home eye injuries occur each year and over 2,000 workers suffer eye injuries each day, many of which are caused by accidental exposure to chemicals or chemical products. Dr. Wind also reviewed the statutes and regulations requiring ocular testing.

Dr. Wind thanked the Panel members for giving their expertise, time, and effort and acknowledged their important role in the ICCVAM test method evaluation process. Dr. Wind also emphasized the importance of public comments that are considered by the Panel in this process and the Panel's role in the development of ICCVAM final test method recommendations.

Welcome from the Director of NICEATM, and Conflict-of-Interest Statements

Dr. Stokes, Director of NICEATM, stated the Panel meeting was being convened as a National Institutes of Health (NIH) Special Emphasis Panel and was being held in accordance with applicable U.S. Federal Advisory Committee Act regulations. As such, Dr. Stokes indicated that he would serve as the Designated Federal Official for this public meeting. He reminded the Panelists that, when they were originally selected, they had signed conflict-of-interest statements in which they identified any potential conflicts of interest. He then read the conflict-of-interest statement and again asked members of the Panel to identify any potential conflicts for the record. Dr. Hayes asked the Panel members to declare any direct or indirect conflicts based on Dr. Stokes' statements and to recuse themselves from voting on any aspect of the meeting where these conflicts were relevant.

Dr. Sawyer declared a potential conflict-of-interest regarding his employment with Minrad Inc., a company that manufactures inhalation anesthetics. Dr. Ward declared a potential conflict-of-interest regarding her consulting relationship with a company that manufactures antimicrobial cleaning products. Dr. Rodeheaver indicated that she worked for Alcon, a manufacturer of the topical anesthetics proparacaine and tetracaine. Dr. Vanparys declared a potential conflict-of-interest regarding his company's involvement in the conduct of the Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) test method.

Overview of the ICCVAM Test Method Evaluation Process

Dr. Stokes opened his presentation by thanking the Panel members for their significant commitment of time and effort preparing for and attending the meeting. He noted that this is an international Panel, made up of 22 different scientists from six different countries (Belgium, Canada, The Netherlands, Japan, Spain, and the United States). He explained that the purpose of the Panel was to conduct an independent scientific peer review of the information provided on several proposed alternative ocular safety test methods, a testing strategy, and proposed refinements to the *in vivo* rabbit eye test method. This assessment is to include an evaluation of the extent that each of the established ICCVAM criteria for validation and regulatory acceptance has been appropriately addressed for each test method or testing strategy. The Panel is then asked to comment on the extent that the available information and test method performance in terms of accuracy and reliability supports the ICCVAM draft recommendations. Dr. Stokes noted that the first ICCVAM Ocular Peer Review Panel met in 2005 to evaluate the validation status of four alternative test methods (Bovine Corneal Opacity and Permeability [BCOP], Isolated Chicken Eye [ICE], Isolated Rabbit Eye [IRE], and the HET-CAM) for their ability to identify ocular corrosives or severe irritants. The Panel recommended two of these test methods (BCOP and ICE) on a case-by-case basis for use in a tiered-testing strategy with test method-specific applicability domain restrictions. ICCVAM and the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) endorsed the Panel's recommended use for these test methods. The Panel also recommended that, while the IRE and HET-CAM test methods were potentially useful in a tiered-testing strategy with appropriate restrictions, additional data were needed to fully assess their usefulness and limitations for regulatory testing. ICCVAM prepared a test method evaluation report (TMER) and provided a transmittal package (i.e., Panel report, SACATM and public comments, TMER and associated materials) to the ICCVAM Federal agencies for their response as required by the ICCVAM Authorization Act of 2000 (ICCVAM 2000). All Federal agencies with ocular testing requirements endorsed the BCOP and ICE test method recommendations. Dr. Stokes noted that five Panel members from the 2005 review are on the current Panel (i.e., Drs. Henry Edelhauser, A. Wallace Hayes, Robert Peiffer, Scheffer Tseng, and Philippe Vanparys).

Dr. Stokes then provided a brief overview of ICCVAM and NICEATM, and identified the 15 Federal agencies that comprise ICCVAM. He summarized the purpose and duties of ICCVAM (as described in the ICCVAM Authorization Act of 2000[2]), noting that ICCVAM, as an interagency committee, does not carry out research and development or validation studies. Instead, ICCVAM, in conjunction with NICEATM, carries out critical scientific evaluations of the results of validation studies for proposed test methods to assess their usefulness and limitations for regulatory testing, and then makes formal recommendations to ICCVAM agencies.

Dr. Stokes then described the ICCVAM test method evaluation process, emphasizing the many opportunities for stakeholder input during numerous public comment periods.

As part of this process, a working group of Federal scientists designated for the relevant toxicity testing area (e.g., the OTWG) and NICEATM prepare a draft background review document (BRD) that provides a comprehensive review of all available data and information. ICCVAM considers all of this available data and information and then develops draft test method recommendations on the proposed usefulness and limitations of the test methods, test method protocol, performance standards, and future studies. The draft BRD and the ICCVAM draft test method recommendations are made available to the Panel and the public for review and comment. The Panel reviews the draft BRD and evaluates the extent to which the established ICCVAM validation and regulatory acceptance criteria have been adequately addressed and the extent that the demonstrated accuracy and reliability support the ICCVAM draft test method recommendations. A Panel report is published and then considered, along with public and SACATM comments, by ICCVAM in developing final recommendations.

[2] http://iccvam.niehs.nih.gov/docs/about_docs/PL106545.pdf

ICCVAM forwards these final recommendations to the ICCVAM member agencies for their consideration and possible incorporation into relevant testing guidelines.

He concluded by summarizing the timeline for 2009 for the ICCVAM evaluation and peer review of the ocular test methods and approaches, including a *Federal Register* notice in March announcing the Panel meeting, the projected publication of the Panel report in July, and transmittal of ICCVAM final recommendations to Federal agencies in November.

ICCVAM Charge to the Panel

Dr. Stokes reviewed the charge to the Panel:

(1) Review the ICCVAM draft BRDs for completeness and identify any errors or omissions (e.g., other relevant publications or available data).

(2) Evaluate the information in the draft BRDs to determine the extent to which each of the applicable ICCVAM criteria for validation and regulatory acceptance of toxicological test methods have been appropriately addressed.

(3) Consider the ICCVAM draft test method recommendations for the following and comment on the extent to which they are supported by the information provided in the BRDs: proposed test method usefulness and limitations, proposed recommended standardized protocols, proposed test method performance standards, and proposed future studies.

Dr. Stokes thanked the OTWG and ICCVAM for their contributions to this project and acknowledged the contributions from the participating liaisons from ECVAM, the Japanese Center for the Validation of Alternative Methods (JaCVAM), and Health Canada. He also acknowledged the NICEATM staff for their support and assistance in organizing the Panel meeting and preparing the review materials.

Overview of the Agenda

Dr. Hayes outlined the process for reviewing each of the topics. First, the test method developer or other expert will describe the test method protocol and procedures, followed by a presentation summarizing the test method validation database and test method performance for each draft BRD or summary review document (SRD) given by a member of the NICEATM staff. An ICCVAM OTWG member will then present the ICCVAM draft test method recommendations. Following presentations, the Evaluation Group Chair responsible for the topic under consideration will present the Evaluation Group's draft recommendations and conclusions followed by Panel discussion. Public comments will then be presented followed by the opportunity for questions to the public commenters and additional Panel discussion. After consideration of the public comments, the Panel will then vote to accept the Panel consensus, with any minority opinions being so noted with a rationale for the minority opinion provided.

Draize Rabbit Eye Test and Current Ocular Regulatory Testing Requirements and Hazard Classification Schemes

Ms. McCall of the U.S. Environmental Protection Agency (EPA) presented the relevant U.S. and international statutes and regulations for ocular safety testing (e.g., EPA, CPSC, Food and Drug Administration [FDA], Occupational Safety and Health Administration [OSHA], European Union [EU], and Organisation for Economic Co-operation and Development [OECD]). She summarized the Draize scoring system for corneal, iridal, and conjunctival lesions in the rabbit, using representative photographs for reference. She also discussed optional but potentially useful assessments of ocular injury (e.g., fluorescein staining, corneal thickness, depth of corneal injury, photographic documentation, and histopathology) that are not routinely included in the Draize eye test. Ms. McCall then provided an overview of the various U.S. and international hazard classification schemes for ocular corrosivity and irritation (i.e., EPA, EU, Globally Harmonized System of Classification and

Labelling of Chemicals [GHS], and Federal Hazardous Substances Act [FHSA]). She noted that, based on the recently adopted European Union Regulation on the Classification, Labelling and Packaging of Substances and Mixtures (i.e., the CLP Regulation), the EU will move to the GHS system after December 1, 2010, for substances and after June 1, 2015, for mixtures. Ms. McCall also identified the required signal words for labeling based on each regulatory classification.

Use of Topical Anesthetics and Systemic Analgesics to Avoid or Minimize Pain and Distress in Ocular Toxicity Testing

On behalf of NICEATM, Dr. Allen reviewed the relevant sections of the draft BRD on the routine use of topical anesthetics and systemic analgesics in *in vivo* ocular irritation testing.

Dr. Merrill then presented the ICCVAM draft recommendations for the routine use of topical anesthetics and systemic analgesics in *in vivo* ocular irritation testing for the Panel to consider.

Panel Evaluation

Dr. Sawyer (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the routine use of topical anesthetics and systemic analgesics in *in vivo* ocular irritation testing and ICCVAM draft test method recommendations. Dr. Sawyer indicated that anesthetic requirements vary enormously among species. For instance, cats require approximately 40% more anesthetic than humans to achieve a similar level of anesthesia. Therefore, any protocol designed to minimize or eliminate pain needs to be individualized to the target species. The Evaluation Group proposed an alternative to the ICCVAM anesthetic/analgesic protocol to be used during all *in vivo* rabbit ocular irritation testing. Dr. Sawyer outlined the Evaluation Group's proposed protocol, which is divided into pretreatment and posttreatment regimens as follows:

Pretreatment Analgesia:
Buprenorphine 0.01 mg/kg subcutaneous (SC) (60 minutes before test substance application [TSA]). Dr. Sawyer noted that buprenorphine is classified as an opioid agonist-antagonist analgesic with a wide margin of safety in rabbits, minimal sedation, and relatively long duration. It has been found to be effective in managing pain in small animals, and is given before application of the test substance because the most effective method of managing pain and distress is to administer the analgesic preemptively to prevent establishment of central sensitization.

One or two drops of 0.5% proparacaine hydrochloride, applied to the eye three times at 5-minute intervals starting 15 minutes pre-TSA. Last application would be five minutes pre-TSA. Anticipated duration of action: 30 - 60 minutes. Dr. Sawyer stated that proparacaine is preferred because application to the eye would be less painful and the suggested application sequence is to assure effective penetration of the epithelial layer.

Eight hours post-TSA:
Buprenorphine 0.01 mg/kg SC and meloxicam 0.5 mg/kg SC. Dr. Sawyer noted that the timing is to reinforce the initial level of analgesia to carry over until the next morning (the duration of analgesia is expected to be at least 12 hours for buprenorphine and at least 24 hours for meloxicam). The combination of an opioid and a nonsteroidal anti-inflammatory drug (NSAID) such as meloxicam is a well-tested approach to balanced analgesia. Used for post-operative or chronic pain in dogs since 1997, meloxicam has been found to have effective application in rabbits.

Day two through day seven post-TSA:
Buprenorphine 0.01 mg/kg SC every 12 hours and meloxicam 0.5 mg/kg SC every 24 hours. Dr. Sawyer noted that buprenorphine and meloxicam should be continued for seven days post-TSA unless signs of ocular injury sufficient to cause pain and discomfort appear. If so, this systemic analgesic protocol would continue until the test is completed.

Rescue Analgesia:
Dr. Sawyer also outlined a procedure where, if a test subject shows signs of physical pain or discomfort during the test interval using the above protocol, a rescue dose of buprenorphine at 0.03 mg/kg SC could be given as needed every eight hours instead of 0.01 mg/kg SC every 12 hours. Meloxicam would continue with the same dose and interval.

Dr. Sawyer pointed out that buprenorphine and meloxicam were synergistic and have an excellent safety profile in clinical practice. A question was raised concerning the interval of dosing throughout the test period and the burden that it would impose on the testing laboratory. The Panel agreed that a ±30-minute interval is appropriate for the administration of the systemic analgesics.

Dr. Dubielzig indicated that the impact of the NSAID on inflammatory aspects of the Draize rabbit eye test is unknown, but the Panel did not consider such affects to be limited and therefore not likely to be a problem. Dr. Jester questioned the need to continue analgesic treatment through day seven when Category III or IV substances would have cleared by day three. He suggested an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) approach where treatment is continued through day four. Dr. Peiffer suggested that the temporal aspect be removed and that treatment be continued only if there are signs of discomfort. **The Panel agreed that treatment should be stopped after day four (instead of day 7, as suggested above) if there are no signs of discomfort**. The Panel agreed that pain assessment should be made and recorded daily.

Dr. Jester raised a concern that the use of preservatives in the topical anesthetics may interfere with the irritation response. The Panel agreed that the use of preservative-free proparacaine should be required. Dr. Stokes asked how long after the administration of the systemic analgesics a rescue dose can be administered. Dr. Sawyer indicated that, due to the wide margin of safety, the rescue dose can be given immediately afterward if necessary.

Dr. Jester expressed concern that dilution of the test substance could occur if a significant amount of liquid anesthetic remained in the eye. Dr. Peiffer indicated that, in his experience, the 5-minute interval is reasonable and should not pose a problem for test substance dilution.

In response to the evaluation guidance question specific to testing situations where the use of topical anesthetics would be considered inappropriate, the Panel indicated that drugs to be used for ocular effects, such as eye drops, need to be tested by other means. However, the focus of this evaluation is eye irritation hazard classification; therefore, the proposal would be relevant to all such testing. The Panel did not know of additional systemic analgesics that might have greater efficacy in relieving ophthalmic pain associated with chemically-induced injuries. The Panel also agreed that there were no additional pain-related chemically-induced injuries to the eye that the proposed alternate analgesic proposal would not adequately address.

The Panel expressed general concern about the use of transdermal patches to deliver anesthetics due to the need for shaving prior to patch application and the possibility of skin irritation. In addition, with multiple applications, the availability of irritation-free skin sites may pose a problem. Most importantly, analgesic patches have proven to be unreliable in clinical practice with significant animal-to-animal variation as well as species-to-species variation when comparing effectiveness and duration of effect. The Panel also indicated a greater concern about self-mutilation due to severe pain during eye irritation testing than about the potential for the systemic analgesics to alter the ocular injury response. Dr. Jester indicated that there was insufficient information in the BRD to make this assessment.

The majority of the Panel agreed that the tetracaine information provided in the ICCVAM BRD could be applied to other topical anesthetics such as proparacaine. Dr. Ward indicated that additional studies on cell proliferation, migration, and cytotoxicity could be done with topical anesthetics to provide some assurance that they behave in a manner similar to tetracaine. Although it was previously noted

that anesthetic/analgesic use was for all *in vivo* eye irritation tests, the Panel indicated that administration of post-application analgesics is not a concern if a standard dosing regimen is used throughout and not adjusted for each animal to avoid overdosing side effects.

The Panel also agreed that the clinical signs of post-application pain and distress are adequately described and that no other clinical signs should be added. In the event of an eye infection, the Panel agreed that secondary treatment should be considered, the signs and symptoms of the eye infection should be documented, and the animal should be immediately removed from the study. Finally, the Panel agreed that all relevant data had been adequately considered in the BRD.

The Panel considered its proposal to be more appropriate than the ICCVAM-proposed recommendations in terms of the type and frequency of dosing for topical anesthetics and systemic analgesics. The Panel agreed with the ICCVAM draft recommendations for future studies. Therefore, it recommended refinement of the current *in vivo* test system to evaluate ocular irritation utilizing contemporary/novel technologies to address both concerns. The Panel recommended the following:

- New animal studies should only be considered when absolutely necessary in developing new strategies for testing.
- Products that are overpredicted when anesthetic and analgesic pretreatment is used should be identified.
- Animal responses should be collected in tests currently being conducted to determine whether refinements are warranted in the dosing and timing of anesthetic, analgesic, and antibiotic treatments.
- Rabbit ocular specimens should be submitted for histopathological evaluation to develop an archive of specimens.
- Digital photographs of lesions/observations should be collected.
- Analysis of the variability in rabbit wound-healing responses would help determine whether or not it is due to variability in the ocular defense linking to the neuroanatomic integration.
- Studies should be conducted to determine whether the timing and dosing of systemic analgesics with topical anesthetics might alter the ocular defense enough to change the classification of test substances.
- Cytology samples from the surface of the eye should be collected.
- Studies should be conducted to investigate the appropriateness of using proparacaine instead of tetracaine.
- Studies should be conducted to evaluate the impact of using the NSAID meloxicam with buprenorphine.
- New technologies (e.g., new imaging modalities and quantitative/mechanistic endpoints) should be incorporated into the Draize rabbit eye test, refining/changing it to make it a more humane test that is also more reliable.

Public Comments

No public comments were made.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion with one abstention, Dr. Rodeheaver, who cited a potential conflict-of-interest due to her employment by a manufacturer of anesthetic products.

Use of Humane Endpoints in *In Vivo* Ocular Irritation Testing

On behalf of NICEATM, Dr. Allen reviewed the relevant sections of the draft BRD on the use of humane endpoints in *in vivo* ocular irritation testing for the Panel.

Dr. Merrill then presented the ICCVAM draft recommendations for the use of humane endpoints in *in vivo* ocular irritation testing for the Panel to consider.

Panel Evaluation

Dr. Sawyer (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the use of humane endpoints in *in vivo* ocular irritation testing and ICCVAM draft test method recommendations. The Panel agreed that each of the current and proposed humane endpoints detailed in the BRD are sufficiently predictive of irreversible or severe effects (i.e., GHS Category 1, U.S. EPA Category I, EU R41) that they should be used routinely as humane endpoints to terminate a study as soon as they are observed. The Panel also agreed that animals should be observed at least once per day (at least twice daily for the first three days) to ensure that termination decisions are made in a timely manner. The Panel agreed that there was insufficient data in the BRD to determine the adequacy of pannus as a recommended humane endpoint. The Panel also agreed that the use of fluorescein staining was an appropriate technique for evaluating eye injury; however, the technique needs to be better described before a reasonable conclusion regarding its value can be made. Dr. Jester suggested that the use of fluorescein staining had not been adequately discussed in this BRD.

The Panel emphasized that, in some cases, decisions to terminate a study should be based on more than one endpoint. Very severe endpoints (e.g., corneal perforation) would be adequate alone to terminate a study. Other biomarkers considered useful by the Panel as routine humane endpoints included extent of epithelial loss, limbal ischemia, and/or stromal loss, and depth of corneal damage.

In response to the question regarding other earlier biomarkers/criteria indicative that painful lesions can be expected to fully reverse, the Panel indicated eyes with conjunctival scores without corneal/iris scores would be expected to recover. The Panel indicated that the destruction of 50% of the limbus will result in pannus in rabbits and, therefore, the ICCVAM draft recommendation requiring 75% for early termination may be excessive. In addition, the Panel indicated that the humane endpoints described in the BRD were sufficient to ensure that the lesions would not reverse. The Panel did agree that the available data and information supported the ICCVAM draft recommendations on humane endpoints. The Panel recommended that studies be developed to identify better and earlier endpoints, such as those seen with fluorescein staining, and that these endpoints should be incorporated into current testing guidelines.

Public Comments

No public comments were made.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion.

Adjournment

Dr. Hayes adjourned the Panel for the day at 5:45 p.m., to reconvene at 8:30 a.m. on Wednesday, May 20, 2009.

WEDNESDAY, MAY 20, 2009

Dr. Hayes called the meeting to order at 8:28 a.m. and asked Dr. Stokes to discuss the conflict-of-interest for the day's planned topics. Dr. Stokes read the conflict-of-interest statement and Dr. Hayes asked the Panel to declare any conflicts-of-interest. The conflicts-of-interest declared by Panel members on day one of the meeting were repeated.

Dr. Hayes then asked for introductions from the Panel, NICEATM staff, members of ICCVAM and the OTWG, and those in attendance for the public session.

HET-CAM Test Method

Dr. Schrage reviewed the various HET-CAM test method protocols (i.e., IS[A], IS[B], S-Score, Q-Score, and IT) and BASF experience with the test method. Dr. Schrage stressed the need for harmonization of HET-CAM protocols, endpoints, and scoring methods. BASF has conducted a retrospective review of 145 test substances, including a broad variety of chemicals and formulations, which revealed that overall accuracy, false positive rates, and false negative rates were not acceptable. The specificity and sensitivity were especially affected by solubility in both water and oil. These data were submitted to the journal Alternatives to Laboratory Animals in April 2009. Dr. Schrage said she would be willing to share the HET-CAM data on these 145 substances with NICEATM following publication.

Dr. Vanparys said that he would be willing to provide NICEATM with HET-CAM data using the IS(B) analysis method to determine if conversion to the IS(A) method was feasible. He added that, in his experience, the HET-CAM test method can be sensitive for the identification of substances not labeled as irritants.

On behalf of NICEATM, Dr. Allen reviewed the HET-CAM draft BRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the HET-CAM test method for the Panel to consider.

Panel Evaluation

Dr. Wilson (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the HET-CAM test method and ICCVAM draft test method recommendations. He noted that HET-CAM classified four EPA Category III substances incorrectly as Category IV (i.e., they were false negative in HET-CAM). However, he said that regulators would be more concerned if the false negative substances were EPA Category I or Category II. Some Panelists did not consider these substances likely to be a significant risk. Dr. Stokes suggested adding a statement defining an acceptable rate for false positives and false negatives. Dr. Wilson expressed concern that, while three of the four animals had an EPA Category III classification that cleared in seven days, one animal had a conjunctival redness score of two that cleared to one in seven days but required 14 days to completely resolve (i.e., return to a score of zero). Such lesions would not be considered inconsequential.

The Panel discussed the low number of mild and moderate substances used in the performance analyses, and that additional substances in these categories would be needed before a conclusion on the usefulness of HET-CAM could definitively be reached. The Panel also recognized that the validation database does not include substances currently regulated by EPA and that collection of additional data is needed. Therefore, given the limited data for mild and moderate substances, the Panel did not support the ICCVAM draft test method recommendation for use of the HET-CAM to identify substances not labeled as irritants from all other classes.

Dr. Peiffer said that he was concerned with the recommendation to test increasing concentrations of test substances. He stated that while dose-response curves are preferred for scientific studies, they are

not practical for regulatory testing. Dr. Sawyer agreed that increasing concentrations should not be a requirement. Ms. McLaughlin argued that use of different concentrations allows the investigator to see if increasing the concentration affects the outcome. She stated that poor predictivity might result from use of a concentration that produces an ineffectual or weak response, whereas the comparative effect of a higher concentration would provide useful information. The Panel agreed to remove the concentration requirement from the test method protocol but to include it as a general recommendation for additional research.

Ms. McLaughlin offered a minority opinion with respect to the Panel's recommendation on the use of the HET-CAM test method to identify substances not labeled as irritants from all other classes. Ms. McLaughlin stressed that personal care products are not regulated in the U.S. as they are in Europe and Canada. Ms. McLaughlin stated that the HET-CAM test method could be used as an alternative to the Draize rabbit eye test to evaluate personal care products in situations where they are regulated. Dr. Hayes asked Ms. McLaughlin to write a short paragraph to note the rationale for her opposition to the majority view for inclusion in the Panel report. Ms. McLaughlin drafted the following text:

Based on the demonstrated performance as outlined in the ICCVAM draft recommendations, HET-CAM can be used to screen not labeled as irritants from other irritant categories for the restricted applicability domain (surfactant-based formulations and oil/water emulsions). The rationale for this dissenting view is based on the fact that there were 60 substances in the overall database. The hazard category distribution was: 25 Category I; 2 Category II; 18 Category III; and 15 Category IV, The sensitivity of HET-CAM is 91% (41/45), resulting in a false negative rate of 9% (4/45). Among the four false negatives for the EPA system, 100% (4/4, all oil/water emulsion cosmetic formulations) were EPA Category III substances based on conjunctival redness score of two that required at least three days to resolve. The lesions noted *in vivo* indicated mild ocular irritation and are unlikely to represent a significant hazard. As such, the HET-CAM could be considered useful as a screening test for EPA Category IV substances not labeled as irritants from all other categories for the restricted applicability domain of surfactant-based formulations and oil/water emulsions. The sensitivity for GHS and EU was high enough for each system to warrant HET-CAM test method use (i.e., 100% sensitivity; 31/31 and 26/26, respectively for GHS and EU [from the ICCVAM draft BRD, Tables 6-2 and 6-12]) also with domain restriction. This performance demonstrates that HET-CAM could be used to screen EU or GHS hazard not labeled as irritant classifications from other irritant categories for the restricted applicability domain of surfactant-based formulations and oil/water emulsions. It should be noted that, for regulatory purposes, sensitivity (the proportion of all positive substances that are classified as positive) is most important from a public health perspective and the HET-CAM performed well in this regard.

The Panel discussed the ICCVAM draft recommended protocol for the HET-CAM test method. Dr. Vinardell said that she would like to see a statement added to the protocol to wash out any leftover solids after 30 seconds (as currently recommended in the EU Annex V). Dr. Hayes asked Dr. Vinardell to provide a statement for Dr. Wilson to include in the Panel report.

The Panel discussed the HET-CAM test method performance. One Panelist suggested that a Chi-square analysis should be included to ensure that differences in classification were statistically significant. Dr. Ahn was asked if a power analysis could be used to determine if the number of substances in the mild and moderate classification was adequate to differentiate the irritant classifications. Dr. Ahn said that there should be at least three substances in each classification category to conduct a power analysis.

The Panel discussed the need for Good Laboratory Practice (GLP) studies. Dr. Hayes emphasized that a study is either GLP compliant or it is not. He said that the phrase "spirit of GLP" should not be used in the Panel report. He also said that the term "original data" should be used rather than "raw data."

The Panel agreed that data from studies not conducted under GLP guidelines could be used to increase knowledge about the applicability domain of a test method but that laboratories should provide sufficient detail about the conduct of the study to understand any deviations from GLP guidelines.

The Panel discussed additional sources of HET-CAM data to expand the applicability domain and the number of mild and moderate substances tested. Dr. Allen noted that Dr. Debbasch, a principal contact for data acquisition, had left L'Oreal. Dr. Hayes said that *cosmeceuticals* represented a gray zone between cosmetics and personal-care formulations, and this class of products should be considered. Ms. McLaughlin said that the inclusion of a single ingredient (e.g., a UV-blocking material) could change the regulatory requirements for a formulation from an unregulated personal care product to a regulated material in Canada. She said that the applicability domain and database used in the ICCVAM draft BRD should be adequate to warrant use of the HET-CAM test method for personal care products that are not labeled as irritants. The Panel did not support the use of additional studies to identify the full range of irritation but supported additional studies to identify substances not labeled as irritants from all other classifications.

Public Comments

Dr. Barroso from ECVAM commented that the false negatives using the EPA classification system, which are substances not labeled as irritants using the GHS classification system, result because the EPA classification system categorizes substances based upon the most severe category observed among the test rabbits (i.e., not based on the majority classification among rabbits tested). Dr. Barroso also said that because the types of formulations regulated by EPA are not present in the database that the EPA classification system should not be given too much weight.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted to approve the recommendations as revised during the discussion with one minority opinion, Ms. McLaughlin, and one abstention, Dr. Vanparys, who cited a potential conflict-of-interest with the HET-CAM test method, which he had worked on at Johnson & Johnson.

Isolated Chicken Eye Test Method

On behalf of NICEATM, Dr. Allen presented an overview of the ICE test method protocol and reviewed the ICE draft BRD. One Panelist asked why the test method was limited to three eyes. Dr. Allen explained that the incubation apparatus contained 10 chambers, sufficient for three groups of three eyes and a negative control. However, the ICCVAM ICE test method protocol, upon which the recently submitted OECD Test Guideline is based, includes both positive and negative controls.

Dr. Jester said that the term fluorescein *staining* should be used rather than *retention*. He also asked how the EPA classification categories were determined using the ICE test method. Dr. Allen replied that the four-tiered EPA classification system was considered equivalent to the four-tiered GHS system and used the same ICE test method decision criteria (e.g., EPA Category I – GHS Category 1, EPA Category II = GHS Category 2A, EPA Category III = GHS Category 2B, EPA Category IV = GHS Category Not labeled).

Dr. Yu asked if the evaluation of the eyes was subjective and whether photographs were taken. Dr. Allen said that the evaluation of the eyes for corneal lesions was subjective, except for the measurement of corneal swelling, which is measured quantitatively using a pachymeter. He said that photographs were not typically taken but were recommended by the previous ocular Panel.

Dr. Merrill then presented the ICCVAM draft recommendations for the ICE test method for the Panel to consider.

Panel Evaluation

Dr. Tarlo (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the ICE test method and ICCVAM draft test method recommendations. The Panel agreed that the available data and test method performance supported the ICCVAM draft recommendations that the ICE test method is not recommended to identify substances from all hazard categories as defined by GHS, EPA, and EU classification systems. The Panel further agreed that the ICE test method is not recommended as a screening test to identify substances not labeled as irritants from all other hazard classifications defined by GHS, EPA, and EU, because one of the false negatives included a GHS Category 1 substance. The Panel agreed with the ICCVAM draft recommendation that the ICE test method should not be used as a screening test to identify GHS substances not labeled as irritants. Dr. van der Valk noted that the ICE test method is used by the Netherlands Organisation for Applied Scientific Research (TNO) to obtain good results, but the results obtained by other laboratories using the ICE test method in the validation study were variable. Dr. Vanparys recommended that the source of the variability be noted in the appropriate text.

The Panel agreed that the available data supported the ICCVAM draft recommendations that the proposed standardized protocol appeared acceptable. However, the Panel suggested that the protocol could be improved by adding objective endpoints for corneal opacity and fluorescein staining. The Panel also added that inclusion of a histopathological evaluation might improve ICE test method performance.

The Panel agreed with the ICCVAM draft recommendations for the ICE test method in terms of the proposed future studies that additional optimization studies would be required to validate the test method for the identification of all ocular irritancy hazard categories. The use of histopathology evaluation might add to the accuracy and determination of the test. The Panel also agreed with ICCVAM that the ICE test method performance standards are not warranted at this time.

Public Comments

Dr. Barroso said that variability of the ICE test method was similar to that of the Draize rabbit eye test because of the subjective assessments. He stated that the ICE test method should not be held to a higher standard than the Draize test. He also noted that the concordance among laboratories was reasonable.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion.

Isolated Rabbit Eye (IRE) Test Method

On behalf of NICEATM, Dr. Allen presented an overview of the IRE test method and reviewed the IRE draft BRD. Dr. Hayes asked whether the rabbits used by GlaxoSmithKline (GSK) were from PelFreeze Biologicals or if fresh eyes were used for each test. Dr. Allen replied that at least some of the rabbits were obtained from other GSK laboratories and had been used as negative controls from other acute safety testing. Dr. Ward noted that PelFreeze ships rabbit eyes from its facility in Rogers, Arkansas, adding that their rabbits are used for multiple purposes. She was not aware of a formal study to determine the acceptability of eyes shipped from the U.S. to Europe. Dr. Peiffer suggested

that shipped eyes should be carefully examined prior to use. Dr. Jester said that his laboratory has compared eyes obtained from an abattoir to fresh eyes and found no significant differences.

Dr. Merrill then presented the ICCVAM draft recommendations for the IRE test method for the Panel to consider.

Panel Evaluation

Dr. Tarlo (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the IRE test method and ICCVAM draft test method recommendations. The Panel agreed with ICCVAM that additional optimization and validation studies using a protocol that includes all four recommended endpoints are needed to further evaluate the relevance and reliability of the IRE test method and to develop more definitive recommendations.

The Panel recommended that the planned validation study with GSK/SafePharm include an evaluation of fresh versus shipped eyes. In general, the Panel felt there should be rigid criteria on the handling and storage of the eyes. Finally, the Panel recommended that criteria on test article administration/washout (e.g., viscous substances) were warranted.

Public Comments

No public comments were made.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion.

Bovine Corneal Opacity and Permeability Test Method (BCOP)

Dr. Curren, Institute for In Vitro Sciences, provided an overview of the BCOP test method. He noted that Pierre Gautheron and his colleagues initially developed the test method for occupational safety. Dr. Curren said that as many as 30% of bovine eyes are rejected upon inspection because of scratches and other defects, and emphasized the importance of including concurrent positive and negative controls in each study. With respect to histopathology evaluation, he said that it was important to carefully choose a qualified laboratory because of the impact of quality on the evaluation.

Dr. Vanparys pointed out that the 15x OD_{490} value in the *In Vitro* Score calculation was chosen to equate the data to *in vivo* data. One Panel member asked if there was an equilibration period, and Dr. Curren indicated that the bovine corneas were equilibrated for one hour before dosing.

Dr. Bailey asked if there was an example for when histopathology evaluation should be recommended based on effects associated with a particular chemical class. Dr. Curren cited as an example oxidizers, which may not produce opacity or permeability changes, but still produce substantive corneal damage that is observable only by histopathology. A Panel member asked why corneal thickness was not measured to provide a quantitative endpoint. Dr. Curren said that corneal thickness has been evaluated, but is less reliable than the opacity and permeability measurements and therefore is not measured in the current protocol.

Dr. Peiffer asked how the BCOP decision criteria for histopathology evaluation are applied to the EPA categorization scheme. Dr. Curren replied that a substance labeled as EPA Category IV would not penetrate further than the superficial corneal epithelium, whereas a Category III substance would penetrate to the basal layer, a Category II substance into the top third of the stroma, and a Category I substance into the bottom third of the stroma or to the endothelium. Minimal damage to the epithelium heals quickly, moderate damage heals more slowly, and significant damage (e.g., deep stromal or endothelial penetration) may be irreversible.

On behalf of NICEATM, Dr. Hamm reviewed the BCOP draft BRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the BCOP test method for the Panel to consider.

Panel Evaluation

Dr. Tarlo (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the BCOP test method and ICCVAM draft test method recommendations. With respect to the substances used in the validation studies, the Panel requested additional chemical classes be added as data becomes available to provide a more significant statistical inference. The Panel requested that Drs. Ahn and Palmer conduct a power analysis to determine the number of substances needed in each hazard classification to provide statistical significance.

The Panel discussed the performance of the BCOP test method to identify the intended range of classification categories. The Panel indicated that the available data and analyses were adequate for the intended purpose. The Panel indicated that all available and relevant data had been used in the ICCVAM BCOP test method analyses.

The Panel agreed with ICCVAM that the test method performance supported the ICCVAM draft recommendations. Accordingly, the BCOP test method was not recommended to identify substances from all hazard categories as defined by GHS, EPA, and EU classification systems. However, the BCOP test method can be used as a screening test to distinguish substances not labeled as irritants from all other hazard categories when results are to be used for EU or GHS hazard classifications. Because of the significant lesions associated with 50% (4/8) of the EPA Category III substances that tested as false negatives, the BCOP test method cannot be recommended as a screening test to identify EPA Category IV substances.

The Panel agreed with the ICCVAM draft recommendation that the BCOP test method could be used to distinguish substances not labeled as irritants from all other irritant classes, because the false negative rate for the EU and GHS systems was 0% (0/54 or 0/97, respectively). By comparison, the false negative rate was 6% (8/141) for the EPA system. Among the eight false negatives for the EPA system, 100% (8/8) were EPA Category III substances based on Draize rabbit eye test data.

The Panel said that, while the BCOP test method is unable to identify all irritant classifications, further test method development and refinement in future studies was encouraged.

The Panel recommended that performance standards should be developed, because the BCOP test method is now being considered as a screening test for both ocular corrosives/severe irritants and for the identification of substances not labeled as irritants.

Public Comments

Dr. Curren said that, based on his experience with the BCOP test method, performance of the BCOP for the four hazard classification systems was unlikely to improve based on the lack of Draize rabbit eye test reproducibility in the mild and moderate categories. He said that results from Weil and Scala (1971) show that the extremes are reproducible, but the mild and moderate levels of ocular irritation are highly variable. He referenced the antimicrobial cleaning products (AMCP) BRD that includes an analysis of the impact on the ocular hazard category when the results of a six-rabbit Draize test are randomly sampled for a three-rabbit test.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Harbell, Mary Kay Inc., said that his laboratories have used over 30,000 bovine eyes that were kept cold at 4ºC. He added that damaged eyes are quickly removed and excluded from the test. He pointed out that Gautheron et al. (1992) used both fresh eyes and eyes maintained at 4ºC and found no differences in their test method results. Dr. Harbell emphasized the utility of the BCOP in comparison to the other methods being considered given its focus on quantitative measurements.

Dr. Harbell also asked the Panel to consider how histopathology evaluation might contribute to the BCOP test method performance. He said that the experts at the 2005 ICCVAM workshop considered the depth of injury to be an important consideration in the assessment of ocular injury. The purpose of including histopathology evaluation is to evaluate the depth of injury that may not be visible to the naked eye. Dr. Harbell cited the example of oxidizing chemicals that may not affect the opacity or permeability of bovine eyes but do still damage the corneal tissue. Therefore, for these substances, depth-of-injury analysis may be important to differentiate corrosives or severe irritants from moderate irritants. Dr. Harbell said he would like to see histopathology evaluation reconsidered. Dr. Ward asked if he was recommending histopathology evaluation for all classes. Dr. Harbell said that he was but that it would be used primarily for EPA Categories I and II.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Barroso commented on what he referred to as the "top-down" (i.e., screening for corrosives/severe irritants) and "bottom-up" (i.e., screening for substances not labeled as irritants) approaches using the ICE and BCOP test methods. ECVAM is developing a paper to recommend the use of these proposed testing strategies for both ICE and BCOP, where substances could be tested in the BCOP or ICE test methods in order to identify corrosives/severe irritants or substances not labeled as irritants without using an animal test.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion (pending the results of a power analysis by Dr. Ahn) with one abstention, Dr. Vanparys, who cited a potential conflict-of-interest with the BCOP test method, which he had worked on at Johnson & Johnson.

Adjournment

After the discussion, Dr. Hayes adjourned the Panel for the day at 7:25 p.m., to reconvene at 8:30 a.m. on Thursday, May 21, 2009.

THURSDAY, MAY 21, 2009

Dr. Hayes convened the Panel at 8:30 a.m. and asked Dr. Stokes to discuss the conflict-of-interest for the day's planned topics. Dr. Stokes read the conflict-of-interest statement and Dr. Hayes asked the Panel to declare any conflicts-of-interest. The conflicts-of-interest declared by Panel members on day one of the meeting were repeated.

Dr. Hayes then asked for introductions from the Panel, NICEATM staff, members of ICCVAM and the OTWG, and those in attendance for the public session.

The first order of business was to address issues from the preceding day.

BCOP Power Calculation

Dr. Ahn reported on the power calculation requested on Wednesday May 20, 2009, for the BCOP test method. He determined that, for each of the four hazard classification systems, a sample size of 13 substances in each chemical class represented (i.e., 13 x 4 for each chemical class for a four-category hazard classification system) is required to achieve 80% power using a two-group normal approximation test for proportions with a one-sided 0.05 significance level. This is necessary to reject the null hypothesis that the BCOP test is inferior to the Draize rabbit eye test (the accuracy of the BCOP test is more than 0.1 less than that of the Draize test) in favor of the alternative hypothesis that the accuracies in the two groups are equivalent. Dr. Ahn also noted that his analysis included the assumption that the expected accuracy of the BCOP test is 0.6 and the expected accuracy of the Draize rabbit eye test is 0.9.

The Panel voted unanimously to include the recommendation that a sample size of 13 be used for each chemical class in each of the four hazard classifications to achieve statistical significance.

ICE Test Method False Negative Substances

Dr. Vanparys commented on the ability of the ICE test method to identify GHS substances not labeled as irritants. Dr. Vanparys indicated that the false negative substances listed in the ICCVAM BRD were either paints that stick to the cornea or solids, which are known to give inaccurate results with the ICE test method. Dr. Vanparys suggested that the ICE test method is capable of identifying GHS substances not labeled as irritants with the exception of solids and substances that stick to the cornea. The overall Panel recommendations, as stated the previous day, remained unchanged.

Low Volume Eye Test (LVET) Test Method

On behalf of NICEATM, Dr. Allen provided a brief overview of the LVET test method and reviewed the LVET draft SRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the LVET for the Panel to consider.

Panel Evaluation

Dr. Sawyer (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the LVET and ICCVAM draft test method recommendations. The Panel noted that the LVET has been used on a wide range of substances and that it does detect the full range of ocular irritancy, but recognized that the majority of the LVET database was for surfactants and surfactant-containing products. The Panel identified several references that should be added to the SRD and noted the need to review the ECVAM BRD. If any additional historical data were obtained, there might be sufficient data to determine the performance of the LVET on several other chemical classes.

The Panel indicated that pain associated with direct application of the test substance to the cornea should not be an issue in light of the recommendations for topical anesthetic and systemic analgesic use.

When discussing the performance of the LVET compared to the Draize test, the Panel indicated that the evaluation was adequate, noting that the LVET appeared to overpredict the human response to a lesser degree than the Draize rabbit eye test. They also recommended that the full range of irritation categories are represented in the LVET validation database.

In considering whether all available data had been made available, the Panel indicated that all data had not been evaluated. Additional published sources should be considered as well as the ECVAM BRD, on which the Panel was unable to comment during this meeting. The Panel stated that in the absence of all existing data, including a background review document prepared by the European Centre for the Validation of Alternative Methods, it could not make definitive conclusions or recommendations on the validation status of the LVET. Nonetheless, the Panel did consider the limited data that are available for the LVET to support the use of historical LVET data as acceptable *in vivo* reference data on which to base comparisons to *in vitro* study results.

Public Comments

Dr. Harbell commented that eye irritation testing is done to protect the public and that accidental exposure data should be included in the evaluation. Dr. Harbell also commented on Dr. Merrill's presentation that outlined the ICCVAM draft recommendations. He stated that the suggestion in the ICCVAM draft recommendations that severe substances should be tested in humans is terrifying. (Note: This comment was in response to a misinterpretation by the commenter, which was clarified by Dr. Merrill who stated that the ICCVAM draft recommendations do not recommend human testing to be conducted [see below]).

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Curren commented that the LVET is being discussed because it was used as an *in vivo* reference test method for some of the data provided for the antimicrobial cleaning product (AMCP) testing strategy. He stated that only biologic or LVET data exist for many of the AMCPs, and these data were used to determine the prediction model to support registration of these AMCPs. The LVET test method is no longer used, but there is historical data that can and should be used. Dr. Curren stated that the question is whether we are putting people at risk based upon the cut-off points suggested in the AMCP BRD.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. McNamee (Procter & Gamble) reiterated the comments by Dr. Curren regarding the LVET and noted that 30 years of human experience data with a chemical substance are sufficient for licensing in the United Kingdom.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Merrill responded to the comment by Dr. Harbell regarding human testing. Dr. Merrill clarified that the ICCVAM draft recommendation states that if an organization or sponsor desires to more adequately characterize the usefulness and limitations of the LVET, ICCVAM recommends that a comprehensive set of substances be tested and compared with the Draize rabbit eye test results. She stated that there was no recommendation for human testing to be conducted, but that existing accidental human injury data and ethical human study data should always be considered.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion with one abstention,

Dr. Ward, who cited a potential conflict-of-interest because of her previous consulting work for a company that conducts the LVET.

Cytosensor® Microphysiometer Test Method

Dr. Curren provided an overview of the Cytosensor Microphysiometer (CM) test method protocol.

On behalf of NICEATM, Dr. Lipscomb reviewed the CM test method performance as detailed in the AMCP draft SRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the CM test method for the Panel to consider.

Panel Evaluation

Dr. Bailey (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the CM test method and ICCVAM draft test method recommendations. The Panel indicated that the test method protocol was sufficiently detailed; however, it was unlikely to be widely used because the CM instrument has been discontinued and a new instrument would require revalidation.

The Panel recommended the use of relevant positive controls in any future validation studies and, because surfactants form micelles that can influence response, surfactant concentrations should be included. The Panel recommended that an evaluation of the different classes of surfactants (i.e., nonionic, anionic, cationic, and zwitterionic) be conducted to determine if restrictions should be imposed on use of the CM test method.

The Panel agreed that, based on the database of surfactants and surfactant-based formulations, LVET data could be used to support the validity of the CM test method in the proposed AMCP testing strategy.

The Panel also agreed that the additional data on the surfactants and surfactant-containing formulations in the ECVAM BRD provided sufficient support for the use of the CM test method as a screening test to identify water-soluble surfactant chemicals and certain types of surfactant-containing formulations (e.g., cosmetics and personal care product formulations but not pesticide formulations) as either severe or corrosive irritants or substances not labeled as irritants in a tiered-testing strategy, as part of a weight-of-evidence approach. The Panel also agreed that the intra- and interlaboratory reproducibility of the CM test method had been adequately evaluated, although for a limited range of substances as previously discussed. The Panel again noted that the instrument has been discontinued and is currently not supported by the manufacturer, making its use difficult. However, if the CM instrument were redesigned, the remanufactured instrument would require "catch-up" validation (i.e., not a full validation study).

Based upon the lesions noted for one false negative substance in the EPA classification system, the Panel expressed concern with the ability of the CM test method to identify EPA Category IV substances. The Panel noted that the rabbit data indicated that this substance would be classified as a Category III and, therefore, may cause irritation in a human. The Panel noted that further CM studies are needed, in particular for EPA Categories III and IV substances.

The Panel also expressed concern with the high false positive rate of the CM test method when identifying all four hazard categories.

Public Comments

Dr. Curren noted a correction to his presentation where he did not specifically state that the CM test method is limited to water-soluble substances. He questioned the need for performance standards for the CM test method, given that the Panel did not recommend performance standards for the BCOP

and ICE test methods. Dr. Curren commented that the surfactants referred to as *personal care products* are really detergents.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion.

EpiOcular Test Method

Dr. Curren provided an overview of the EpiOcular (EO) test method protocol.

On behalf of NICEATM, Dr. Lipscomb reviewed the EO test method performance as detailed in the AMCP draft SRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the EO test method for the Panel to consider.

Panel Evaluation

Dr. Bailey (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the EO test method and ICCVAM draft test method recommendations. The Panel agreed that the EO test method protocol is adequately detailed but emphasized that the manufacturer should provide a "certificate of quality" for each batch of EO. The Panel also agreed that the critical aspects of the protocol had been justified and described in the BRD; however, in order to use the EO test method in a testing strategy to identify mild irritants and substances not labeled as irritants, positive controls that represent these hazard categories should be included in any future validation studies. The Panel noted that the EO test method cannot distinguish Category III from Category IV substances.

The Panel commented that the performance of the EO test method had not been adequately evaluated and compared to the Draize test for the types of substances included in the AMCP database. The Panel noted that the total number of products and their distribution across hazard categories were not sufficient. The Panel commented that the intralaboratory variability was not adequately assessed, although interlaboratory variability was considered to be adequate.

Public Comments

Dr. Curren indicated that he felt that it was appropriate to include EO data that used a different protocol as a measure of test method reproducibility.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion with one abstention, Dr. Ward, who cited a potential conflict-of-interest because of her previous consulting work for a company that conducts the EO test method.

Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products (AMCPs) Using *In Vitro* Alternative Test Methods

Dr. Curren provided an overview of the AMCP testing strategy.

On behalf of NICEATM, Dr. Lipscomb reviewed the AMCP draft SRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the AMCP testing strategies for the Panel to consider.

Panel Evaluation

Dr. Bailey (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the AMCP testing strategies and ICCVAM draft test method recommendations. The Panel also suggested adding more discussion of the cells used in the CM and EO test methods.

Regarding the BCOP test method, the Panel reflected on its previous discussions of the BCOP test method for the total database. The Panel indicated that use of the BCOP test method in a testing strategy to identify severe irritants (Category I) and moderate irritants (Category II), should include positive controls that represent these hazard categories in any future validation studies. The Panel noted that histopathology evaluation, as it is proposed at this time as an additional endpoint for the BCOP test method, does not justify its use for hazard classification of AMCPs. However, histopathology evaluation may prove to be a useful endpoint and, as such, collection of histopathology data and further efforts to optimize its use are encouraged.

The Panel agreed with the ICCVAM draft recommendations that there is insufficient data to support the testing strategy in terms of the proposed test method usefulness and limitations (i.e., the classification of substances in all four ocular hazard categories). There were also insufficient available data on which to base definitive recommendations on the proposed alternate testing strategy for classifying substances in all four ocular hazard categories. In discussing the validity of retrospective evaluations, the Panel stated that a retrospective evaluation of results could be considered adequate if the studies were performed with GLP compliance, coded samples, and pre-established evaluation criteria. The Panel commented that any definitive recommendations on a testing strategy should be based on prospective testing of a list of reference substances in each of the proposed *in vitro* test methods.

The Panel concurred with the ICCVAM draft recommendations in terms of the proposed test method standardized protocols. The Panel stated that routine fixation of tissue from the BCOP test method for possible histopathology evaluation should be continued. The Panel emphasized that no single *in vitro* test method alone was applicable to all types of test materials, and therefore suggested several future studies that could potentially expand the usefulness of AMCP test strategies.

Finally, the Panel commented that the development of performance standards for the AMCP testing strategy was not currently warranted and that a new approach needed to be defined for comparing testing strategies.

Public Comments

Dr. Barroso commented that ECVAM is working on a guideline for the detection of severe irritants with the BCOP test method. He indicated that they see a small change in classification when the cut-off is changed from 55 to 75. ECVAM considers 55 the best cut-off for their intended purpose.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Curren commented that concern regarding the limited number of AMCPs is misplaced due to the intended narrow applicability domain. He stated that industrial-strength cleaners are mostly severe irritants and that household cleaners are mostly mild irritants. Very few, if any, substances are in the moderate range. Dr. Curren expressed concern with the recommendation by the Panel that substances need to be tested by each test method in the testing strategy. He noted that histopathology evaluation with the BCOP test method was included in the testing strategy to provide additional safety, and clarified that most of the histopathology evaluation was performed by a certified veterinary

pathologist. He also questioned the Panel's suggested use of a transformed ocular cell line rather than a normal epidermal cell line.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion with one abstention, Dr. Ward, who cited a potential conflict-of-interest because of her previous consulting work for a company that manufactures AMCPs.

Concluding Remarks

Dr. Hayes, on behalf of the Panel, thanked Dr. Stokes and the NICEATM staff for their continued assistance during the review process and Panel meeting. He also thanked Dr. Wind, ICCVAM Chair, and the members of ICCVAM and the OTWG for their contributions to the project. Finally, Dr. Hayes thanked the Panel and the Evaluation Group Chairs.

Drs. Wind and Stokes thanked the Panel again for their hard work, thoughtful and objective deliberations, and advice. Dr. Stokes further thanked public attendees for their participation and the invited test method developers for their excellent test method summaries. Dr. Stokes concluded by saying he looked forward to working further with Panel members to complete the Panel report.

Adjournment

Dr. Hayes adjourned the Panel at 7:40 p.m., concluding the meeting.

William S. Stokes, D.V.M., D.A.C.L.A.M.
NIEHS
P.O. Box 12233
Mail Stop: K2-16
Research Triangle Park, NC 27709

Dear Dr. Stokes,

The Meeting Summary Minutes, Independent Scientific Peer Review Panel Meeting, Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches, accurately summarizes the Peer Review Panel Meeting on May 19-21, 2009, in Bethesda, MD.

Sincerely,

/s/ A. Wallace Hayes 8/28/09

Signature | Printed Name | Date

Appendix C2

Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches

The full document is available electronically on the enclosed CD-ROM or at:
http://iccvam.niehs.nih.gov/docs/ocutox_docs/OcularPRPRept2009.pdf

The document is also available on request from NICEATM:

NICEATM
National Institute of Environmental Health Sciences
P.O. Box 1233, MD K2-16
Research Triangle Park, NC 27709 USA
Telephone: 919-541-2384 Fax: 919-541-0947
E-mail: niceatm@niehs.nih.gov

This page intentionally left blank

Independent Scientific Peer Review Panel Report:
Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches

July 2009

Interagency Coordinating Committee on the
Validation of Alternative Methods

National Toxicology Program Interagency Center for the
Evaluation of Alternative Toxicological Methods

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
Department of Health and Human Services

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

This document is available electronically at
http://iccvam.niehs.nih.gov/docs/ocutox_docs/OcularPRPRept2009.pdf

The findings and conclusions of this report are those of the Independent Scientific Peer Review Panel and should not be construed to represent the official views of ICCVAM or its member agencies.

When referencing this document, please cite as follows:

Interagency Coordinating Committee on the Validation of Alternative Methods. 2009. Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches. Research Triangle Park, NC: National Institute of Environmental Health Sciences.

Table of Contents

List of Figures

List of Abbreviations and Acronyms

AHT	Animal health technician
AMCP	Antimicrobial cleaning product(s)
BCOP	Bovine corneal opacity and permeability
BRD	Background review document
CEC	Commission of the European Communities
CM	Cytosensor Microphysiometer®
CPSC	U.S. Consumer Product Safety Commission
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EC/HO	European Commission/British Home Office
ECVAM	European Centre for the Validation of Alternative Methods
EO	EpiOcular™
EPA	U.S. Environmental Protection Agency
EU	European Union
FDA	U.S. Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GHS	Globally Harmonized System of Classification and Labeling of Chemicals
GLP	Good Laboratory Practice
HET–CAM	Hen's egg test – chorioallantoic membrane
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICE	Isolated chicken eye
IRE	Isolated rabbit eye
IS	Irritation score
ITC	Irritation threshold concentration
IVIS	*In vitro* irritancy score
LVET	Low volume eye test
NEI	National Eye Institute
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NICNAS	National Industrial Chemicals Notification and Assessment Scheme (Australia)

NIH	National Institutes of Health
NSAID	Nonsteroidal anti-inflammatory drug
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OSHA	U.S. Occupational Safety and Health Administration
OTWG	ICCVAM Ocular Toxicity Working Group
SC	By subcutaneous injection
SRD	Summary review document
TG	Test Guideline
TSA	Test substance application
UN	United Nations
V1	First branch of the trigeminal nerve

[This Page Intentionally Left Blank]

Members of the Independent Scientific Peer Review Panel

A. Wallace Hayes, Ph.D., DABT, FATS, ERT (Panel Chair), Visiting Scientist, Harvard School of Public Health, Andover MA; Principal Advisor, Spherix Inc., Bethesda, MD

Hongshik Ahn, Ph.D., Professor, Department of Applied Mathematics and Statistics, Stony Brook University, Stony Brook, NY

Paul T. Bailey, Ph.D., Bailey and Associates Consulting, Neshanic Station, NJ

Richard Dubielzig, D.V.M., Professor, School of Veterinary Medicine, University of Wisconsin—Madison, Madison, WI

Henry Edelhauser, Ph.D., Professor of Ophthalmology and Director of Ophthalmic Research, Emory University School of Medicine, Atlanta, GA

Mark Evans, D.V.M., Ph.D., DACVP, Pathology Lead for Ophthalmology Therapeutic Area, Pfizer Global Research and Development at La Jolla Drug Safety Research and Development, San Diego, CA

James V. Jester, Ph.D., Professor of Ophthalmology and Biomedical Engineering, University of California—Irvine, Orange, CA

Tadashi Kosaka, D.V.M., Ph.D., Associate Director—Toxicology Division, Chief, Laboratory of Immunotoxicology and Acute Toxicology, The Institute of Environmental Toxicology, Ibaraki, Japan

Alison McLaughlin, MSc, DABT, Environmental Impact Initiative, Office of Science and Risk Management, Health Products and Food Branch, Health Canada, Ottawa, Canada

J. Lynn Palmer, Ph.D., Associate Professor, Dept. of Palliative Care and Rehabilitation, University of Texas M.D. Anderson Cancer Center, Houston, TX

Robert Peiffer, Jr., D.V.M., Ph.D., DACVO, Senior Investigator, Safety Assessment, Merck Research Laboratories, West Point, PA

Denise Rodeheaver, Ph.D., DABT, Director, Department of Toxicology, Alcon Research Ltd., Fort Worth, TX

Donald Sawyer, D.V.M., Ph.D., DACVA, HDABVP, Professor Emeritus, College of Veterinary Medicine, Michigan State University, East Lansing, MI

Kirk Tarlo, Ph.D., DABT, Scientific Director, Comparative Biology and Safety Sciences, Amgen, Inc., Thousand Oaks, CA

Daryl C. Thake, D.V.M., DACVP, Midwest ToxPath Sciences Inc., Chesterfield, MO

Scheffer Tseng, M.D., Ph.D., Director, Ocular Surface (OS) Center, Medical Director OS Research & Education Foundation; Director R&D Department, Tissue Tech, Inc., Miami, FL

Jan van der Valk, Ph.D., Senior Scientist, Department of Animals, Science and Society, Faculty of Veterinary Medicine, Netherlands Centre Alternatives to Animal Use, Utrecht University, Utrecht, Netherlands

Philippe Vanparys, Ph.D., Managing Director, CARDAM (VITO), Mol, Belgium

Maria Pilar Vinardell, Ph.D., Director, Department of Physiology, Professor of Physiology and Pathology, Universitat de Barcelona, Barcelona, Spain

Sherry L. Ward, Ph.D., MBA, In Vitro Toxicology Consultant, BioTred Solutions; Science Advisor, International Foundation for Ethical Research, New Market, MD

Daniel Wilson, Ph.D., DABT, Mammalian Toxicology Consultant, Toxicology and Environmental Research Consulting, The Dow Chemical Co., Midland, MI

Fu-Shin Yu, Ph.D., Director of Research, Department of Ophthalmology & Anatomy, School of Medicine, Wayne State University, Detroit, MI

Preface

The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) convened an international independent scientific peer review panel (hereafter, Panel) meeting on May 19-21, 2009 at the U.S. Consumer Product Safety Commission Headquarters in Bethesda, MD. The Panel, which included 22 expert scientists from six countries, evaluated test methods and approaches that may further reduce and refine the use of animals for ocular safety testing.

These evaluations included the following:

- A proposal for the routine use of topical anesthetics, systemic analgesics, and humane endpoints to avoid and minimize pain and distress during *in vivo* ocular irritation testing
- The use of the bovine corneal opacity and permeability (BCOP), the Cytosensor Microphysiometer® (CM), the isolated chicken eye, the isolated rabbit eye, and the hen's egg test – chorioallantoic membrane test methods for identifying moderate and mild ocular irritants and substances not labeled as ocular irritants
- The *in vivo* low volume eye test
- Nonanimal testing strategies that use the BCOP, CM, and/or EpiOcular™ test methods to assess the eye irritation potential of antimicrobial cleaning products and determine their appropriate U.S. Environmental Protection Agency ocular hazard classification

During the May 2009 public meeting, the Panel discussed each test method and approach, listened to public comments, and developed conclusions and recommendations for ICCVAM. The Panel emphasized its consideration in the following areas: (1) review of the ICCVAM draft background review documents (BRDs) for completeness and identification of errors or omissions of existing relevant data or information that should be included, (2) evaluation of the information in the draft summary review documents (SRDs) and BRDs to determine the extent to which each of the applicable ICCVAM criteria for validation and acceptance of toxicological test methods had been appropriately addressed, and (3) consideration of the ICCVAM draft test method recommendations and comment on the extent to which they are supported by the information provided in the draft BRDs or SRDs for the following:

- Proposed test method uses and limitations
- Proposed recommended standardized protocols
- Proposed future studies

This report details the Panel's independent conclusions and recommendations. ICCVAM will consider this report and all relevant public comments as it develops final test method recommendations. The ICCVAM final test method recommendations will be forwarded to U.S. Federal agencies for their consideration in accordance with the ICCVAM Authorization Act of 2000 (Public Law 106-545).

The Panel gratefully acknowledges the efforts of NICEATM staff in coordinating the logistics of the Panel meeting and in preparing materials for its review. The Panel also appreciates the participation of Drs. Rodger Curren and Arnhild Schrage in the meeting by providing descriptions of several of the test method protocols being considered. Finally, as Panel Chair, I want to thank each Panel member for her or his thoughtful and objective review of these test methods and approaches.

A. Wallace Hayes, Ph.D., DABT, FATS, FIBiol, FACFE, ERT

Chair, Alternative Ocular Safety Testing Methods Peer Review Panel

July 2009

Executive Summary

This report describes the conclusions and recommendations of an international independent scientific peer review panel (hereafter, Panel). The Panel was charged by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) with evaluating the validation status of several proposed test methods and testing approaches. These include:

- A proposal for the routine use of topical anesthetics, systemic analgesics, and humane endpoints to avoid or minimize pain and distress during required *in vivo* ocular irritation safety testing
- Five individual *in vitro* test methods for identifying ocular irritants, including the bovine corneal opacity and permeability (BCOP), Cytosensor Microphysiometer® (CM), isolated chicken eye (ICE), isolated rabbit eye (IRE), and the hen's egg test – chorioallantoic membrane (HET-CAM) test methods
- The *in vivo* low volume eye test (LVET), proposed as an alternative to the current *in vivo* rabbit eye test
- Nonanimal testing strategies using three *in vitro* test methods (the BCOP, CM, and EpiOcular™ [EO] test methods) to assess the eye irritation potential of antimicrobial cleaning products (AMCPs) for U.S. Environmental Protection Agency (EPA) ocular hazard classification and labeling purposes

The Panel evaluated the validation status of each proposed test method and testing strategy according to established Federal and international criteria (ICCVAM 1997, OECD 2005). The Panel also commented on ICCVAM draft recommendations regarding the usefulness and limitations of each proposed test method and testing strategy.

Use of Topical Anesthetics and Systemic Analgesics to Minimize Pain and Distress in Ocular Toxicity Testing

The Panel agreed with the ICCVAM draft recommendation that topical anesthetics and systemic analgesics should routinely be used for *in vivo* ocular toxicity studies to avoid or minimize pain and distress. The Panel differed with the ICCVAM draft recommendation on the most appropriate protocol for using topical anesthetics and systemic analgesics in ocular toxicity testing procedures. The Panel proposed an alternative preemptive pain management protocol for all *in vivo* rabbit eye irritation tests intended for regulatory safety testing, unless there is a requirement for monitoring the pain response (e.g., pharmaceutical tolerability testing).

The Panel also recommended that pain assessments should be made immediately after test substance application and recorded daily (i.e., at least twice daily, or more often as necessary).

Use of Humane Endpoints to Minimize Pain and Distress in Ocular Toxicity Testing

The Panel concluded that, based on the available data and information, some humane endpoints as recommended by ICCVAM are adequate to terminate a study. The Panel concluded that the current and proposed humane endpoints are predictive enough of irreversible or severe effects (United Nations Globally Harmonized System of Classification and Labeling of Chemicals [GHS] Category 1, EPA Category I, European Union [EU] R41) that they should routinely be used as humane endpoints to terminate a study as soon as they are observed. However, the Panel emphasized that, while very severe endpoints (i.e., corneal perforation) would be adequate alone to terminate a study, determinations to terminate a study should typically be based on more than one endpoint.

The Hen's Egg Test – Chorioallantoic Membrane Test Method

The Panel agreed with the ICCVAM draft recommendation that, based on an evaluation of available data and corresponding performance (e.g., overall correct classifications that ranged from 40% [23/58] to 41% [24/59]), the HET-CAM test method is not recommended to identify substances from all hazard categories as defined by the GHS (UN 2007), EPA (EPA 2003a), and EU (EU 2001) classification systems.

The Panel did not support the ICCVAM draft recommendation (with one minority opinion) that based on the available data, the HET-CAM IS(A) test method can be used as a screening test to identify substances as not labeled as irritants from all other hazard categories when results are to be used for EU or GHS hazard classifications. The Panel concluded that there were too few surfactants or oil/water emulsions in the mild to moderate irritant categories to have sufficient confidence in the ability of the test to distinguish them from the not labeled as irritant category. However, the Panel did identify possible sources of other existing data that could be analyzed, and they recommended reconsideration of the test method following appropriate analyses.

One Panel member expressed a minority opinion that based on the demonstrated performance, HET-CAM should be recommended to screen substances not labeled as irritants from all other irritant categories for the restricted applicability domain (surfactant-based formulations and oil/water emulsions) for the GHS, EU and EPA hazard classification systems. This Panel member also noted that, for regulatory purposes, sensitivity (the proportion of all positive substances that are classified as positive) is most important from a public health perspective and the HET-CAM performed well in this regard.

The Isolated Chicken Eye Test Method

The Panel supported the draft ICCVAM recommendations that, based on an evaluation of available data and corresponding performance (e.g., overall correct classifications for ICE test method ranged from 59% [83/141] to 77% [118/153]), the ICE test method is not recommended to identify substances from all hazard categories as defined by GHS, EPA and EU classification systems. The Panel also agreed that, based on false negative substances that include at least one substance classified as an ocular corrosive/severe irritant based on Draize rabbit eye data (n = 1 each for the EPA and GHS systems, and n = 6 for the EU system), the ICE test method is not recommended as a screening test to identify substances not labeled as irritants from all other hazard categories as defined by GHS, EPA, and EU classification systems.

The Isolated Rabbit Eye Test Method

The Panel agreed with the ICCVAM draft recommendations that, based on the lack of a standardized protocol and insufficient data using all four recommended IRE endpoints, additional studies are needed before definitive recommendations on the relevance and reliability of the IRE test method can be made.

The Bovine Corneal Opacity and Permeability Test Method

The Panel supported the draft ICCVAM recommendations for the BCOP test method that, based on an evaluation of available data and corresponding performance (e.g., overall correct classifications that ranged from 49% [91/187] to 54% [101/186]), the test method is not recommended to identify substances from all hazard categories as defined by GHS, EPA, and EU classification systems.

The Panel also concluded that the BCOP test method can be used as a screening test to identify substances not labeled as irritants from all other hazard categories when results are to be used for EU or GHS hazard classifications. However, due to the significant lesions associated with 50% (4/8) of the EPA Category III substances that were false negative in the BCOP test method, the BCOP test method cannot be recommended as a screening test to identify EPA Category IV substances.

The Low Volume Eye Test

The Panel concluded that in the absence of all existing data, including a background review document prepared by the European Centre for the Validation of Alternative Methods, it could not make definitive conclusions or recommendations on the validation status of the LVET. Nonetheless, the Panel did consider the limited data that are available for the LVET to support the use of historical LVET data as acceptable *in vivo* reference data on which to base comparisons to *in vitro* study results.

The Cytosensor Microphysiometer® Test Method

The Panel concluded that the available data and performance support the ICCVAM draft recommendations on usefulness and limitations for the CM test method. The Panel concluded that the CM test method can be used as a screening test to identify both ocular corrosive/severe irritants and substances not labeled as irritants, but this use is limited to water-soluble surfactant chemicals and specific types of surfactant-containing formulations (e.g., cosmetics and personal care products). The Panel expressed concern about the availability of the instrument used to conduct the CM test method.

Antimicrobial Cleaning Products Testing Strategies

The Panel agreed with the ICCVAM draft recommendations that there were insufficient data to support the use of the proposed AMCP testing strategy (i.e., using the BCOP, CM, and EO test methods) for classification of substances in all four EPA ocular hazard categories. The Panel also agreed with the ICCVAM draft recommendations that there were insufficient available data on which to base definitive recommendations on an alternate testing strategy (i.e., using the BCOP and EO test methods) for classifying substances in all four EPA ocular hazard categories.

The Panel commented that the absence of data on substances tested in all three *in vitro* test methods (i.e., BCOP, CM, and EO) prevented any definitive recommendation on the AMCP testing strategy. In addition, the availability of only *in vivo* LVET data for some test substances complicated evaluation of *in vitro* test method performance. The Panel recommended that additional EPA-registered AMCPs representing all ocular hazard categories, in particular EPA Categories II and III, be examined in all tests involved in the proposed strategy.

The Panel recognized that the use of histopathological evaluation as an additional endpoint did not improve the accuracy and predictability of the BCOP test method for the limited database of currently tested AMCPs. However, histopathological evaluation may eventually prove to be a useful endpoint, and as such collection of ocular tissue for possible histological evaluation, as well as further efforts to optimize the use of histopathology as an endpoint in BCOP, is recommended.

Appendix B
Peer Review Panel Member Biosketches

[This Page Intentionally Left Blank]

Panel Member Biosketches

Hongshik Ahn, Ph.D.

Dr. Ahn received a Ph.D. in statistics with a minor in computer sciences from the University of Wisconsin–Madison. He is a Professor in the Department of Applied Mathematics and Statistics at Stony Brook University in New York. He has been a Visiting Scientist at the National Center for Toxicological Research at the U.S. Food and Drug Administration (FDA) since 1997 and a Senior Biostatistician for the General Clinical Research Center at Stony Brook since 2005. His research interests include tree-structured regression and classification, survival analysis, bioinformatics, generalized linear model, animal carcinogenicity studies, toxicology, and risk assessment. Dr. Ahn is Associate Editor for *Communications in Statistics* and a member of the International Biometric Society (Eastern North American Region) and the American Statistical Association. He is a referee for 18 statistical journals including *Journal of the American Statistical Association*, *Biometrics*, *Statistics in Medicine*, and *Risk Assessment*. In 2005, Dr. Ahn participated in the National Institutes of Health (NIH) Biostatistical Methods and Research Design Study Section. He has published three book chapters, 48 peer-reviewed publications, 21 proceedings, and has received 11 special invitations to serve as conference session chair or invited speaker.

Paul T. Bailey, Ph.D.

Dr. Bailey received his Ph.D. in psychopharmacology from Howard University. He is currently a consultant for Bailey & Associates Consulting in Neshanic Station, New Jersey. Dr. Bailey also has served as a toxicology consultant with expertise in clinical research; quality assurance (Good Laboratory Practice [GLP] and Good Clinical Practice); chemical exposure and health hazard and/or risk assessment; product liability; technical expertise; regulatory toxicology related to chemicals, petroleum products, cosmetics, personal health care, medical device, and household product industries; strategic planning and management of product safety evaluation and toxicological research programs that are needed to meet industry and regulatory requirements. Dr. Bailey is a former Senior Research Associate at Mobil Oil Corporation with expertise in the development and use of *in vitro* methods to assess the potential eye and skin irritation or sensitization potential of petroleum products and in the validation of alternative methods. At Proctor & Gamble, he was a Divisional Toxicologist (Group Leader) and supervised the dermal toxicology laboratory that focused on development of protocols and in-house or contract laboratory testing to assess the toxicology of potential personal care products. Dr. Bailey has served on numerous government scientific advisory panels (Federal Insecticide, Fungicide and Rodenticide Act [FIFRA], National Institute of Environmental Health Sciences, National Toxicology Program

[NTP]) and trade organizations (e.g., The Cosmetic, Toiletry and Fragrance Association, Chemical Manufacturer's Association). He was a member of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Immunotoxicology Working Group and served on the Editorial Board of the *Journal of the Dermal Clinical Evaluation Society*. Dr. Bailey has contributed to 45 publications or meeting abstracts.

Richard Dubielzig, D.V.M.

Dr. Dubielzig received his Ph.D. from the University of Minnesota. Dr. Dubielzig is currently Professor of Pathology in the School of Veterinary Medicine at the University of Wisconsin–Madison. His primary research interests are comparative dental pathology and comparative ophthalmic pathology. Dr. Dubielzig is an honorary Diplomate of the American College of Veterinary Ophthalmologists (ACVO). He has trained over 40 postdoctoral residency or clinical instructor candidates in pathology or ophthalmology. Dr. Dubielzig is a member of the Central Committee of the Comparative Ophthalmic Research Laboratories, a collaborative research team that provides clinical, pathology, and basic science support to industry in the development of ocular compounds and evaluation of ocular toxicity. Dr. Dubielzig is a member of numerous professional and scientific organizations including the American Veterinary Medical Association, the American College of Veterinary Pathologists, the Association for Research in Vision and Ophthalmology, the Society of Toxicologic Pathologists, the International Society of Ocular Toxicology, and the International Society of Veterinary Ophthalmology. Dr. Dubielzig has authored or coauthored over 198 articles in peer-reviewed journals, 17 book chapters, and 259 abstracts. He has been invited to give 119 lectures.

Henry F. Edelhauser, Ph.D.

Dr. Edelhauser obtained his Ph.D. in physiology from Michigan State University. He is a Professor of Ophthalmology, Director of Ophthalmic Research, and Adjunct Professor of Biology at Emory University. Dr. Edelhauser is the Program Director of the National Eye Institute Research Training Grant "Multidisciplinary Training in Vision Research" at Emory University. His major research interests include physiological mechanisms of corneal transparency; role of sulfhydryls on corneal endothelial function; corneal permeability and cellular toxicity of intraocular irrigating solutions, drugs, and enzymes; the physiological effects of vitrectomy on ocular tissues; dynamics of intraocular fluids, ocular toxicology, corneal extracellular matrix, corneal endothelial physiology; corneal effects of eicosanoids and other lipid mediators; schlera permeability; and cellular mechanisms of ocular inflammation. He has served as chair of the Cornea Section of the Association for Research in Vision and Ophthalmology; chaired or participated in several National Eye Institute or

other NIH Study Sections, workshops, and Special Emphasis Panels; and serves on various editorial boards for eye research journals. Dr. Edelhauser has authored or coauthored 292 publications in peer-reviewed journals, contributed to 51 books or book chapters and four audiotapes, and given 29 lectures or invited talks as a visiting professor. In 2005, Dr. Edelhauser was an active participant on the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)-ICCVAM Expert Panel to review the current validation status of four *in vitro* test methods for identifying ocular corrosives and severe irritants.

Mark Evans, D.V.M., Ph.D., DACVP

Dr. Evans received his D.V.M. and Ph.D. degrees from Michigan State University. He is the Pathology Lead for Ophthalmology Therapeutic Area in Drug Safety Research and Development at Pfizer Global Research and Development in La Jolla, California. Dr. Evans is on the Adjunct Clinical Faculty in the Department of Pathology, College of Veterinary Medicine at Michigan State University and serves as the point of contact for the Michigan State University/Pfizer cosponsored residency program. He is chair of the Corporate Partners Subcommittee of the American College of Veterinary Pathologists. He has 27 journal publications and 38 abstracts. He is a Diplomate of the American College of Veterinary Pathologists, the Society of Toxicologic Pathologists, the United States and Canadian Academy of Pathology, and the American Veterinary Medical Association.

A. Wallace Hayes, Ph.D., DABT, FATS, ERT

Dr. Hayes received his Ph.D. in biochemistry from Auburn University. He is a Principal Advisor for Spherix Incorporated in Bethesda, Maryland. Dr. Hayes is also a Research Professor in the Department of Pharmacology and Toxicology at the Medical College of Virginia in Richmond and an Adjunct Professor in the School of Veterinary Medicine at the Virginia Polytechnical Institute in Blacksburg, Virginia; the Department of Physiology and Pharmacology at Wake Forest University in Winston-Salem, North Carolina; and the Department of Pharmacology and Toxicology at the University of Louisville School of Medicine. Dr. Hayes is a Diplomate of the American Board of Toxicology, a registered regulatory toxicologist (ERT) for EUROTOX, and a Fellow of the American Toxicological Society in addition to being a member of a number of professional specialty boards. He holds a variety of editorial posts for journals including *Cutaneous and Ocular Toxicology*, *Toxicology and Applied Pharmacology*, *Regulatory Toxicology and Pharmacology*, and *Food and Chemical Toxicology*. Dr. Hayes has served on many advisory and expert panels for U.S. and international regulatory interests, including NICEATM-ICCVAM, and for risk assessment, health and safety, or toxicological interests. He has served on various task groups

and scientific advisory boards. He is a reviewer for 28 journals. He is a course director for Principles of Toxicology at the Harvard School of Public Health. Dr. Hayes has authored or coauthored 200 publications in peer-reviewed journals, 11 books, 73 invited presentations, nearly 100 invited seminars, and 152 abstracts presented at scientific meetings. Dr. Hayes is a member of numerous professional societies including the Society of Toxicology, the International Society of Regulatory Toxicology and Pharmacology, the American Society of Pharmacology and Experimental Therapeutics, the American College of Toxicology, and the American Society of Quality Control.

James V. Jester, Ph.D.

Dr. Jester received his Ph.D. in the Department of Pathology at the University of Southern California Medical Center in Los Angeles. Dr. Jester is a Professor of Ophthalmology and Biomedical Engineering at the University of California, Irvine, where he is the Jack H. Skirball Endowed Chair. Dr. Jester is a recognized international leader in the cell biology of corneal wound healing, a research field on which he has had a major impact. Dr. Jester is a member of numerous review boards for ocular pathology and eye irritation. He is an *ad hoc* reviewer for the National Eye Institute (NEI) VISA 1 (Vision Sciences A) and Small Business Innovation Research Study Sections and a reviewer on the Anterior Eye Disease Study Panel of the NEI. He has participated in numerous ocular workshops and symposia including the ICCVAM Ocular Symposia on Ocular Mechanisms held at the NIH in Bethesda, Maryland, in 2005 and the European Cosmetic, Toiletry and Perfumery Association workshop on Eye Irritation Alternatives held in Brussels in 2008. Dr. Jester participates on the editorial boards of eight ocular journals including *Investigative Ophthalmology & Visual Science*, *Experimental Eye Research*, *Cutaneous and Ocular Toxicology*, *Cornea*, and *Current Eye Research*. He also serves on various program-planning committees for ocular research and biology. Dr. Jester is a member of the American Association for the Advancement of Science, the New York Academy of Science, the Association for Research in Vision and Ophthalmology, the American Society for Cell Biology, the International Congress on Eye Research, and the International Society for Ocular Cell Biology. Dr. Jester has published 202 peer-reviewed manuscripts, 14 nonrefereed publications, 223 abstracts, and 45 invited presentations.

Tadashi Kosaka, D.V.M., Ph.D.

Dr. Kosaka received his D.V.M. and Ph.D. degrees from the School of Veterinary Medicine at the Nippon Veterinary and Animal Science University. He is Associate Director and Chief of the Laboratory of Immunotoxicology and Acute Toxicology in the Toxicology Division in The Institute of Environmental Toxicology in Ibaraki, Japan. His research, which covers the

areas of immunotoxicology and acute toxicology, is represented in 24 publications in peer-reviewed journals. Dr. Kosaka is a member of the Japanese Association for Laboratory Animal Science, the Japanese Society of Toxicology, the Japanese Society of Immunotoxicology, and the Japanese Society of Alternatives to Animal Experiments.

Alison McLaughlin, MSc., DABT

Ms. McLaughlin received her Master's Degree in biology from Queen's University in Kingston, Ontario, Canada. A Diplomate of the American Board of Toxicology (2004), Ms. McLaughlin is a Senior Science Policy Analyst for the Environmental Impact Initiative in the Office of Science and Risk Management, Health Products and Food Branch of Health Canada in Ontario. Ms. McLaughlin was formerly a Toxicologist/Senior Evaluator and Acting Section Head in the New Substance Assessment and Control Bureau on Notifications for Food and Drug Products. In this capacity, she developed experience and interest in alternative test methods such as the hen's egg test – chorioallantoic membrane and the bovine corneal opacity and permeability test methods. Ms. McLaughlin served as an editor for the Parliament of Canada on the House of Commons Standing Committee on Environment and Sustainable Development to produce a year 2000 report on pesticides that included information on human health impacts, environmental impacts, and contaminants in the traditional diet of northern communities. Ms. McLaughlin has 17 publications, including results of several Canadian government-sponsored environmental impact studies.

J. Lynn Palmer, Ph.D.

Dr. Palmer received her Ph.D. in biometrics from the University of Texas Health Science Center, Houston. Dr. Palmer has a joint appointment as Associate Professor (Tenured) in the Department of Palliative Care and Rehabilitation Medicine–Research, Division of Cancer Medicine and Associate Professor of Biostatistics in the Department of Biostatistics and Applied Mathematics at the University of Texas M.D. Anderson Cancer Center. Dr. Palmer is a member of numerous professional and scientific organizations. These include the American Statistical Association, of which she served as a chair, a member of numerous committees, and as president of the local Houston chapter. She is also a member of the International Biometrics Society, the Royal Statistical Society, the International Society for Bayesian Analysis, the International Association of Hospice & Palliative Care, and the American Society of Clinical Oncology. Dr. Palmer has authored or coauthored 139 articles in peer-reviewed journals, plus seven additional publications (reviews, letters to editors, etc.) and five book chapters. Dr. Palmer has organized or chaired nine symposia or conferences and presented at 38 national and international scientific conferences.

Robert L. Peiffer, Jr., D.V.M., Ph.D., DACVO

Dr. Peiffer received a D.V.M. degree and a Ph.D. in comparative ophthalmology from the University of Minnesota, St. Paul. He is a Senior Investigator at the Merck Research Laboratories, Adjunct Professor of Ophthalmology at the Scheie Eye Institute at the University of Pennsylvania, Emeritus Professor of Ophthalmology and Pathology at the University of North Carolina in Chapel Hill, and Director of Bucks County Animal Ophthalmology. He has been a consultant in ophthalmology and comparative ophthalmic toxicology for several major pharmaceutical and eye care companies, medical schools, and zoological parks and animal preserves. Dr. Peiffer is on the review boards of 16 journals and is a contributing editor for several others. He has served on several committees for the National Academy of Sciences, the National Institutes of Health, a FIFRA Scientific Advisory Panel, and an ICCVAM Expert Panel (2005). Dr. Peiffer has published 152 articles in refereed journals, with three more in submission; 70 articles in nonrefereed journals; 9 book reviews; nearly 160 papers and presentations at scientific meetings; and numerous visiting professorships and lectureships in the U.S. and around the world. Dr. Peiffer is a member of the American Academy of Ophthalmology, the American Society of Veterinary Ophthalmology, the International Society of Ophthalmology, the International Society of Ocular Toxicology, and the International Society of Ophthalmic Pathology, among others.

Denise Rodeheaver, Ph.D., DABT

Dr. Rodeheaver received her Ph.D. in toxicology from the University of Georgia. She is currently Director of the Toxicology Department at Alcon Research, Ltd., in Fort Worth, Texas. Dr. Rodeheaver is responsible for the qualitative and quantitative achievements of Consumer Products Toxicology and *In Vitro* Toxicology, and oversight of Toxicology Compliance. Dr. Rodeheaver has experience in acute, subchronic, and chronic toxicity evaluations (e.g., ocular and systemic toxicity, genotoxicity, sensitization) conducted in-house or at contract research organizations. She is Diplomate of the American Board of Toxicology, a member of the Society of Toxicology, and Sigma Xi. Dr. Rodeheaver is currently a board member for the International Society of Ocular Toxicology. Dr. Rodeheaver has 13 publications in peer-reviewed journals, 13 abstracts or posters presented at scientific meetings, and 18 presentations at scientific meetings including the International Society of Ocular Toxicology Congress and the Association for Research in Vision and Ophthalmology annual meeting.

Donald C. Sawyer, D.V.M., Ph.D., DACVA, HDABVP

Dr. Sawyer received a Doctorate in Veterinary Medicine from Michigan State University and a Ph.D. in anesthesia and surgery at the Surgery Laboratory Advanced Degree Program at

Colorado State University. He is a member of the Scientific Advisory Board and a Manager of Veterinary Development for Minrad International. Dr. Sawyer was a Captain in the U.S. Air Force serving as a support surgeon at the School of Aerospace Medicine. Dr. Sawyer is Professor Emeritus in the College of Veterinary Medicine at Michigan State University. He served on the faculty of Michigan State University as Professor of Anesthesia, Coordinator of Lifelong Education and Alumni Affairs, and researcher on anesthesiology and pain assessment in cats and dogs. He is a founding member of the American College of Veterinary Anesthesiologists and cofounder of the American Board of Veterinary Practitioners. Dr. Sawyer is a council member and Secretary/Treasurer of the World Congress of Veterinary Anaesthesiology. He has been elected to two six year terms as a member of the American Veterinary Medical Association Council on Biologic and Therapeutic Agents and served as chair for 3 years. Dr. Sawyer has published nine books/monographs, two textbooks, 22 chapters, 68 scientific articles, and 94 abstracts/proceedings. He has had 210 invited papers and presentations.

Kirk Tarlo, Ph.D., DABT

Dr. Tarlo received a Ph.D. from the Rackham Graduate School at the University of Michigan. He is Scientific Director, Comparative Biology and Safety Sciences, at Amgen, Inc., in Thousand Oaks, California. Dr. Tarlo is former Scientific Director, Toxicology, at Allergan, Inc., in Irvine, California. His research interests include toxicology, *in vitro* cytotoxicity, safety evaluation, genetic toxicology, and regulatory issues relating to investigational new drugs and new drug applications. Dr. Tarlo has 11 publications in refereed journals and has given 18 presentations at professional/scientific meetings. He is a Diplomate of the American Board of Toxicology and a member of the Environmental Mutagen Society, the Society of Toxicology, and the Southern California Society of Toxicology.

Daryl Thake, D.V.M., DACVP

Dr. Thake received a D.V.M. from Iowa State University in Ames, Iowa. He is board certified by the American College of Veterinary Pathologists. Dr. Thake is the president and owner of Midwest ToxPath Science, Inc., and was a principal and co-owner of Seventh Wave Pathology and Biotechnical Solutions in Chesterfield, Missouri. Dr. Thake held numerous leadership roles in toxicology and pathology at Pharmacia and its legacy companies, Searle and Monsanto. He was a Senior Science Fellow and Global Head of Pathology Sciences at Pharmacia Corporation in St. Louis, Missouri, where he was responsible for the in-house and CRO pathology functions across five sites in the U.S. and Europe. As the Head of Carcinogenicity Assessment, Global Pathology Sciences, Dr. Thake developed experience in

pathology laboratory techniques including immunohistochemistry, *in situ* hybridization, laser capture microscopy, and imaging. As a consulting pathologist, his work involves gross and microscopic pathology evaluation of preclinical toxicology studies in support of drug discovery and development. He is also involved in the design and conduct of studies for management of toxicology issues in response to regulatory agency concerns with target products. He has been particularly involved in peer reviews to identify and resolve pathology issues and/or problems. Dr. Thake is a member of the Society of Toxicologic Pathologists, American College of Veterinary Pathologists, and the American Veterinary Medical Association. He serves on the editorial board of the *American Journal of Veterinary Pathology*. He is past chairman of the Scientific and Regulatory Policy Committee, Society of Toxicologic Pathologists, and past chairman and current member of the Government Policy Committee of the American College of Veterinary Pathologists. Dr. Thake has 23 publications in peer-reviewed journals.

Scheffer Chuei-Goong Tseng, M.D., Ph.D.

Dr. Tseng received his M.D. degree from the National Taiwan University Medical School and his Ph.D. degree in experimental pathology from the Department of Pathology, University of California, San Francisco, Medical Center. He was board certified by the American Board of Ophthalmology. Dr. Tseng is Director of the Ocular Surface Center; Research Director of the Ocular Surface Research & Education Foundation; Medical Director and Consultant for Bio-Tissue, Inc.; Director of Research and Development of TissueTech, Inc.; and a Board Director for MedNet, Inc. He is an adjunct investigator in the Division of Medical Engineering at the National Health Research Institute in Taiwan and has served on various NIH committees as an *ad hoc* member. His research interests include ocular surface biochemistry and biology, reconstruction and surgical procedures for limbal epithelial stem cell transplantation for total limbal deficiency. Dr. Tseng has published 30 books, 193 peer-reviewed journal manuscripts, and a large body of other works, publications, abstracts, and presentations. Dr. Tseng also has six invention disclosures and holds 12 U.S. or Taiwanese patents or provisional patents. He serves as a reviewer for 28 journals including *Ophthalmology*, *American Journal of Ophthalmology*, *The Lancet*, *New England Journal of Medicine*, *Journal of Refractive Surgery*, and *Gene*. He serves on the editorial board of six journals including *Ocular Surface*, *Cornea*, and *Investigative Ophthalmology Visual Sciences*. Dr. Tseng is a member of 19 professional societies including the American Medical Association, Association for Research in Vision and Ophthalmology, and the American Academy of Ophthalmology.

Jan van der Valk, Ph.D.

Dr. van der Valk received a Ph.D. from the Australian National University in Canberra. He is a Senior Scientist at the Netherlands Centre for Alternatives to Animal Use in the Department for Animals, Science & Society of the Faculty of Veterinary Medicine at Utrecht University. Dr. van der Valk is the Dutch representative on the European Centre for the Validation of Alternative Methods (ECVAM) Scientific Advisory Committee (ESAC). He has served on several other committees involved in evaluation and review of alternative toxicological methods including the ESAC Shadow Review Panel (chair) of the Joint ICCVAM/ECVAM validation study on organotypic assays, INVITTOX (2004, 2006), the Congress on Alternatives held at the University of Linz, Austria (2006, 2008), and the European Society of Toxicology In Vitro (ESTIV; 2008). Dr. van der Valk also serves as Secretary of ESTIV and of INVITROM (Dutch-Belgian Society for *In Vitro* Methods). Dr. van der Valk was a board member of ecopa (European consensus-platform for alternatives).

Phillipe A. Vanparys, Ph.D

Dr. Vanparys received his Ph.D. with Greatest Distinction from the Catholic University of Louvain in Belgium. He is the Managing Director of the Centre for Advanced Research & Development on Alternative Methods (CARDAM) in Mol, Belgium. He was formerly a Senior Research Fellow and Head of Genetic and *In Vitro* Toxicology at Johnson and Johnson Pharmaceutical Research & Development (J&J) in Beerse, Belgium. Dr. Vanparys was a representative for J&J (Beerse) on the J&J Research & Development Committee for *In Vitro* Alternatives. He was also an Industrial Representative in the Belgian Platform for Alternative Methods and serves as a representative for the pharmaceutical industry in the Structure Working Group and Technical Working Group of the Foundation for Alternatives to Animal Testing. Dr. Vanparys also serves as a nominated test method expert on the Genotoxicity/Mutagenicity and the Eye Irritation subgroups for ECVAM to establish timetables for phasing out animal testing as required by the 7th Amendment to the Cosmetics Directive (2003/15/EC). Dr. Vanparys serves as Chairman of the Expert Group on Cell Transformation testing and as a member of the Expert group on *in vitro* micronucleus testing and the Carcinogenicity Taskforce at ECVAM. He is the Belgian representative in the Organisation for Economic Co-operation and Development Task Force on the application of GLP principles to *in vivo* studies. He also served on an ICCVAM Expert Panel for Ocular Corrosives. Dr. Vanparys holds numerous professional memberships including the European and Belgian Environmental Mutagen Societies, member of and auditor for the Belgian and European Toxicology Societies, the European Society of Toxicology In Vitro, the Environmental Mutagen Society, and the *In Vitro* Testing Industrial Platform. Dr. Vanparys has 44 publications, with three in preparation, and three international reports. He has also

contributed to several hundred confidential internal reports, reviews, and expert reports for Janssen Research Foundation and J&J Research and Development.

Maria Pilar Vinardell, Ph.D.
Dr. Vinardell is currently Director of the Department of Physiology and Professor of Physiology and Physiopathology in the Faculty of Pharmacy at the University of Barcelona. Dr. Vinardell teaches *in vitro* toxicology courses in various Latin American countries including Argentina, Cuba, Chile, and Brazil. A registered toxicologist (Spain and EUROTOX), Dr. Vinardell is responsible for the research group "Interaction of surfactants and cell membranes." She was responsible for and has conducted more than 500 *in vitro* and *in vivo* studies on preclinical toxicology for cosmetic, pharmaceutical, veterinary, and chemical industries since 1978. These studies include skin and eye irritation, acute toxicity, subacute toxicity, subchronic toxicity, sensitization, pyrogens, intramuscular irritation, assessment of analgesic and anti-inflammatory activities, histology, and interleukin determinations. Dr. Vinardell has experience in writing standard operating procedures for risk assessment. She is actively involved in research in alternatives to eye and skin irritation and to the rabbit pyrogen test. She has collaborated with and provided draft scientific reports to ECVAM and other research centers. Dr. Vinardell is a peer reviewer for 17 journals and has provided public comment and submitted material on several ICCVAM-related activities. She has given over 100 presentations or invited lectures at national and international congresses. Dr. Vinardell has 90 publications in peer-reviewed journals, 12 review articles, 6 book or educational publications, and 12 books by invitation.

Sherry Ward, Ph.D., MBA
Dr. Ward received her Ph.D. in biochemistry from Michigan State University, an MBA from the University of Maryland University College (UMUC), and an executive M.S. in Technology Management from UMUC. She currently consults for BioTred Solutions in New Market, Maryland. Dr. Ward has expertise in *in vitro* toxicology, scientific/technical/business writing and communication, research and project management, grant proposal review, and grant writing. She also has experience in market research, commercialization, and strategy development and is a contributing editor to AltTox. Dr. Ward is an adjunct faculty member in Biotechnology & Project Management at UMUC. She has animal welfare experience. As a Staff Scientist and *In Vitro* Toxicology Laboratory Manager at the Gillette Company, she developed, characterized, and drafted patent applications for the first human conjunctival epithelial cell lines and gained experience in bioassay development and validation. Dr. Ward has served on numerous scientific panels and committees and was a panel member and presenter at the ICCVAM symposia on mechanisms of ocular injury and recovery and minimizing pain and distress in ocular toxicity testing held at NIH in 2005. She has been

actively involved with trade organizations and served on the European Cosmetic, Toiletry and Perfumery Association Eye Irritation Task Force and the International Life Sciences Institute–Health and Environmental Sciences Institute Alternatives to Animals Task Force. Dr. Ward's experience in models of eye irritation and mechanisms of injury is reflected in 19 publications in peer-reviewed journals, four unpublished validation or prevalidation documents related to ICCVAM activities, 17 presentations, 28 abstracts, and a patent. She is a member of the Hopkins Medical and Surgical Association and the Washington Academy of Sciences.

Daniel M. Wilson, Ph.D., DABT

Dr. Wilson received his Ph.D. in biochemistry/toxicology from Michigan State University. He is currently a Mammalian Toxicology Consultant in Toxicology for Environmental Research and Consulting at the Dow Chemical Company in Midland, Michigan. Dr. Wilson is a board-certified toxicologist with expertise in mammalian toxicology, genetic toxicology, genetic polymorphisms, *in vitro* alternatives, biochemistry, nutritional biochemistry, FDA-regulated food-contact toxicology, and medical device toxicology. He has technical experience in risk assessment for Dow operations and products, for risks associated with intermediates used for contract pharmaceutical formulations, and for characterization of health risks to workers and consumers. Dr. Wilson also has responsibility for the identification and facilitation of testing for particular products and assesses data requirements for setting appropriate occupational exposure and manufacturing limits. Dr. Wilson provides expert business assistance in the area of environmental health and safety to Dow businesses, toxicological review of the chemistry and products within the business, and international registration activity. He participates in trade associations relevant to business activities and is an active member of the Animal Welfare Opportunity Team. Dr. Wilson has published 18 articles in peer-reviewed journals, 1 book chapter, and 30 abstracts. He is a Diplomate of the American Board of Toxicology. Dr. Wilson is a member of the Society of Toxicology and past president and Secretary of the Midwest Regional Chapter. He was a member of the 2006 NICEATM-ICCVAM Expert Review Panel for Alternatives to Acute Toxicity Testing and served on several animal welfare, ISO standardization, biosafety, and radiation safety committees.

Fu-Shin Yu, Ph.D.

Dr. Yu received his Ph.D. from Wayne State University. Dr. Yu is currently Professor and Director of Research at the Kreske Eye Institute in the Department of Ophthalmology, Department of Anatomy and Cell Biology at the Wayne State University School of Medicine. He was an Associate Professor at the Schepens Eye Institute at Harvard University. Dr. Yu is

a member of the Association for Research in Vision and Ophthalmology. He serves as a reviewer for four ocular research journals and for 10 other journals or organizations (e.g., the Wellcome Trust). Dr. Yu currently receives funding for studies on the molecular regulation of corneal wound healing, modulation of epithelial barrier function during corneal infection, and mechanisms of flagellin-induced protection against bacterial keratitis. Dr. Yu has published 59 articles in peer-reviewed journals and three book chapters or review articles; he was an invited speaker or presenter at 17 seminars or ocular research meetings. A participant on state and local boards and committees, Dr. Yu is also an editorial board member of the *Journal of Toxicology–Cutaneous and Ocular Toxicology* and a member of the National Scientific Advisory Council and the American Federation for Aging Research.

Appendix D

ECVAM Scientific Advisory Committee (ESAC)

Statement on the Use of Existing Low Volume Eye Test (LVET) Data for Weight of Evidence Decisions on Classification and Labelling of Cleaning Products and Their Main Ingredients

This page intentionally left blank

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
In vitro methods Unit
European Centre for the Validation of Alternative Methods (ECVAM)

STATEMENT ON

THE USE OF EXISTING LOW VOLUME EYE TEST (LVET) DATA FOR WEIGHT OF EVICENCE DECISIONS ON CLASSIFICATION AND LABELLING OF CLEANING PRODUCTS AND THEIR MAIN INGREDIENTS

At its 31st meeting, held on 7 and 8 July 2009, the non-Commission members of the ECVAM Scientific Advisory Committee (ESAC) unanimously endorsed the following statement:

1. The ESAC strongly recommends that the Low Volume Eye Test (LVET) method, a modification of the standard Draize eye test, is NOT conducted in the future to generate new testing data concerning the intrinsic properties of xenobiotic substances (chemicals, cosmetic ingredients etc.).

2. ESAC nevertheless acknowledges that existing[1] LVET data of the limited use domain of household detergents and cleaning products as well as their main ingredient class (i.e. surfactants as used in these products) may be used for purposes of classification and labelling decisions.

3. Moreover, existing LVET data of this limited use domain may be used as supplementary data in the context of a subset of future validation studies.

4. Finally, the ESAC recommends that no additional testing is done to further develop or validate the LVET test method.

The ESAC recommends that consideration be given on a case by case basis to the limited use of existing Low Volume Eye Test (LVET) data as supplementary in vivo data within Weight of Evidence (WoE) evaluations of alternative testing methods and strategies, and for decision making on the necessity to conduct additional standard in vivo test method(s) for eye irritation for purposes of classification and labelling for the above specified limited use domain.

This recommendation is based on conclusions reached following the assessment of a dossier submitted to ECVAM concerning data and test results relating to detergents, cleaning products and, to a lesser extent, their main ingredients (surfactants).

In making these recommendations, ESAC acknowledges:

(1) the considerable amount of existing LVET data for the domain of household detergents and cleaning products;

(2) that the LVET makes use of direct corneal exposure to mimic specific human exposure scenarios that can be reasonably expected (e.g. accidental ocular exposure during household use) and for the specific use domain of household detergents and cleaning products as well as their main ingredients (i.e. surfactants) as used in these products.

[1] Existing data in this context refers to data that were generated ***prior*** to the date of this statement.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
In vitro methods Unit
European Centre for the Validation of Alternative Methods (ECVAM)

(3) that LVET data, being based on exposure scenarios likely to be relevant in humans, may predict effects in humans with improved accuracy when compared to the Conventional Calculation Method (CCM) traditionally used for C&L decisions on products of this use domain;

(4) the provisions of the Regulation on the Classification, Labelling and Packaging of Substances and Mixtures ('CLP' Regulation 1272/2008/EC; Ref. 1), which foresees an WoE assessment based on existing data to determine whether or not testing with accepted standard tests (i.e. those described in the Test Method Regulation 440/2008/EC; Ref. 2) is necessary or may be dispensed with.

The ESAC furthermore recognises that several databases for alternative methods for eye irritation test methods may be of an acceptable size only if existing LVET testing data can be considered as an additional and secondary source of supporting information. Some differences in classification based on LVET data are to be expected with respect to reference data for the established eye irritation test (i.e. Draize eye data), and the tendency of them to give lower hazard categories than the classical Draize eye test (Ref. 3) must be kept in mind. Nevertheless these data may still be useful on a case by case basis, and only with respect to testing data for household detergents, cleaning products and surfactants used in such products. Subject to these considerations existing LVET data may on occasion contribute to a knowledge base against which alternative methods may be validated for this specific use domain.

Joachim Kreysa

Head of Unit

In-Vitro Methods Unit

European Centre for the Validation of Alternative Methods

Ispra, 9. July 2009

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
In vitro methods Unit
European Centre for the Validation of Alternative Methods (ECVAM)

Explanatory background to this ESAC recommendation:

This recommendation is based on a submission of LVET data to ECVAM concerning household detergents and cleaning products as well as their main ingredient class, i.e. surfactants. The LVET data were correlated to effects in man observed in response to accidental splashes and as documented in poison control centres and clinics. To a lesser extent also clinical exposure data from human volunteers on substances of the mild irritant range were used. The submission was evaluated by ECVAM in 2006 and, after requested amendments had been performed, underwent independent ESAC peer review from April 2007 to June 2009.

The LVET is a minor modification of the classical Draize eye irritation test (Ref. 4): the LVET differs from the Draize test only with regard to two aspects both relating to exposure: (1) The LVET uses only a tenth of the volume of liquids (=10µL) or weight of solids (=10mg) in comparison to the Drazie (0.1 mL of liquids and 100mg of solids); (2) both liquids and solids are applied directly on the cornea in the LVET, without subsequent forced closure of eyelids, in contrast to the Draize test where the test material is instilled in the conjunctival sac of the rabbit eye. All other parameters such as e.g. exposure time and visual scoring of effects on the cornea, conjunctiva and iris are unchanged with regard to the Draize eye test. All other parameters such as e.g. exposure time and visual scoring of effects on the cornea, conjunctiva and iris are unchanged with regard to the Draize test. The rationale for using a reduced amount of test substances (as described in the submission) and for applying it directly to the cornea is to mimick household exposure scenarios such as accidental splashes with detergents and cleaning products in man and to consequently approximate the effects in man. The ESAC PRP held that while such exposure scenarios may be reasonable specifically for household detergents and cleaning products they do not take into consideration other possible routes of exposure such as, for instance, the accidental exposure to pesticides using pressure pumps during field work. Thus, while the LVET exposure settings may be appropriate for household exposure to cleaning products and, possibly, personal hygiene products (i.e. cosmetics), they do at present not appear appropriate for a wide range of substances and associated exposure scenarios – at least until further data supporting such use becomes available.

The LVET has been and is used mainly by industry to benchmark finished products (formulations = mixtures= preparations), a blend of individual chemical substances purposefully mixed in measured and defined proportions for specific uses and applications (e.g. cleaning products, shampoos etc.). In practice, LVET data were used to contribute to classification and labelling decisions. Up to January 2009, when the new CLP regulation came into force (see below), classification and labelling of substances was performed according to the Dangerous Substance Directive (Directive 67/548/EEC; Ref. 5) and that of mixtures according to the Dangerous Preparations Directive (Directive 1999/45/EC; Ref. 6). In December 2008 the EU adopted the Regulation on the Classification, Labelling and Packaging of Substances and Mixtures (so-called CLP regulation 1272/2008/EC; Ref. 1) that aligns existing EU legislation to the United Nations Globally Harmonised System (GHS). The CLP Regulation will, after a transitional period, replace the current rules on classification, labelling and packaging of substances (Directive 67/548/EEC; Ref. 5) and mixtures (Directive 1999/45/EC; Ref. 6). The date from which classification and labelling must be consistent with the new rules will be 1 December 2010 for substances and 1 June 2015 for mixtures. Notably,

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
In vitro methods Unit
European Centre for the Validation of Alternative Methods (ECVAM)

the CLP regulation amends the REACH regulation (concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals, 'REACH'; 1907/2006/EC, Ref. 7) with respect to classification and labelling.

Both, the CLP regulation and REACH foresee the possibility of WoE assessments to decide on the necessity of standard tests to be performed (i.e. tests laid down in the Test Method Regulation 440/2008/EC; Ref. 2): the CLP regulation in the context of classification and labelling decisions of substances and mixtures (formerly referred to as 'preparations' or 'formulations'; these may include finished products for consumer use) and REACH in the context of chemical safety assessments.

WoE approaches are based on the integration of data from various sources and make use of synergistic effects obtained by combining data sets in cases where each single data on its own would be insufficient for decision-making but where the combination of data may allow conclusions on the absence or presence of dangerous properties of substances as regulated by the CLP regulation and the REACH regulation and finished products (i.e. "mixtures", previously referred to as "preparations"), as regulated by the CLP regulation.

LVET data related to above mentioned use domain may be helpful, together with other existing and available data from various sources, to decide in a WoE approach in the contexts of the two above mentioned regulations whether confirmatory standard test(s) for eye irritation are necessary or whether existing information, in its totality, is sufficient to arrive at classification and labelling conclusions without performing further testing of the substance/product in question.

In summary the ESAC recommendation takes into consideration:

(a) the non-negligible amount of human reference data collected in the submitted dossier;

(b) the fact that LVET data, as the classical Draize eye test, reflect these human exposure data at least for above mentioned and limited use domain (e.g. detergents/cleaning products and surfactants) (see however point c)

(c) the fact that most of the exposed patients had received anti-inflammatory treatment which complicates an appraisal to which extent observed effect in patients represented the actual hazard to be expected under observation of the precautionary principle;

(d) the appraisal that the exposure settings of the LVET may represent the exposure from accidental splashes more appropriately than the classical Draize eye test;

(e) the common practices concerning the labelling of finished products under the Dangerous Preparations Directive (Ref. 6) as well as the future practice using WoE evaluations for substance and product classification and labelling as outlined in the CLP regulation (Ref. 1);

(f) the fact that the LVET is only a very minor variation of the Draize test with no impact on i) the amount of animals required for testing and ii) with unknown effects for the test animals with regard to the extent of stress and suffering inflicted.

(g) the potential usefulness of existing LVET data as reference data for validation purposes of alternative methods to assess the ocular irritancy potential of raw materials (surfactants) and finished products of the use domain of detergents and cleaning products.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
In vitro methods Unit
European Centre for the Validation of Alternative Methods (ECVAM)

(h) the comparable reproducibility of the LVET when compared to he Draize eye test.

REFERENCES

1. European Union (2008). Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006). *Official Journal of the European Union* L 353/1.

2. European Union (2008). Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) *Official Journal of the European Union* L 142/51

3. Cormier EM, Parker RD, Henson C, Cruse LW, Merritt AK, Bruce RD, Osborne R (1996) Determination of the intra- and interlaboratory reproducibility of the low volume eye test and its statistical relationship to the Draize eye test. *Regul Toxicol Pharmacol.* **Apr;23(2):**156-61.

4. Draize, J.H., Woodard, G. & Calvery, H.O. (1944) Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. and Exp. Therapeutics.* 82, 377–390.

5. European Communities (1967) Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances *Official Journal of the European Union* 196 , 16/08/1967 P. 0001 – 0098

6. European Communities (1999) Directive 1999/45/EC of the European Parliament and of the Council of 31 May 1999 concerning the approximation of the laws, regulations and administrative provisions of the Member States relating to the classification, packaging and labelling of dangerous preparations. *Official Journal of the European Communities* L 200/1

7. European Union (2006). Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. *Official Journal of the European Union* L 396/1.

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
In vitro methods Unit
European Centre for the Validation of Alternative Methods (ECVAM)

The ESAC was established by the European Commission, and is composed of nominees from the EU Member States, industry, academia and animal welfare organisations, together with representatives of the relevant Commission services.

This statement was endorsed by the following members of the ESAC:

Ms Argelia Castaño(Spain)
Ms Maija Dambrova (Latvia)
Ms Alison Gray (ESTIV)
Ms Katalin Horvath (Hungary)
Ms Dagmar Jírová (Czech Republic)
Mr Roman Kolar (Eurogroup for Animals)
Ms Elisabeth Knudsen (Denmark - acting as moderator at the meeting)
Mr Manfred Liebsch (Germany)
Mr Gianni Dal Negro (EFPIA)
Mr. Walter Pfaller (Austria)
Mr Tõnu Püssa (Estonia)
Mr Dariusz Sladowski (Poland)
Mr Jon Richmond (UK)
Ms Vera Rogiers (ECOPA)
Mr Michael Ryan (Ireland)
Ms Annalaura Stammati (Italy)
Mr Jan van der Valk (The Netherlands)
Mr Carl Westmoreland (COLIPA)
Mr Timo Ylikomi (Finland)

The following Commission Services and Observer Organisations were involved in the consultation process, but not in the endorsement process itself:

Commission services
Mr Joachim Kreysa (DG JRC, Head of In vitro methods Unit/ECVAM, chairman)
Mr Claudius Griesinger (DG JRC, ESAC secretariat)
Ms Susanne Hoke (DG ENTR)
Ms Susanna Louhimies (DG ENV)
Mr Juan Riego Sintes (DG JRC)

The following observers were present
Mr Hajime Kojima (JaCVAM)
Mr William Stokes (NICEATM)
Ms Marilyn Wind (ICCVAM)

Appendix E

Federal Register Notices and Public Comments

This page intentionally left blank

Appendix E1

Federal Register Notices

This page intentionally left blank

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

National Toxicology Program; National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); Request for Data on Non-Animal Methods and Approaches for Determining Skin and Eye Irritation Potential of Antimicrobial Cleaning Product Formulations; Request for Nominations for an Independent Expert Panel

AGENCY: National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH).

ACTION: Request for data and nomination of panelists.

SUMMARY: The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and NICEATM are requesting the submission of data that would assist in evaluating the validation status of non-animal methods and approaches used for determining the skin and eye irritation potential of antimicrobial cleaning product formulations to meet regulatory hazard classification and labeling purposes. Additionally, NICEATM is also requesting the nomination of scientists for consideration as potential members of an independent scientific expert panel (''Panel'') to evaluate the proposed methods and approaches. The ICCVAM will consider the conclusions and recommendations from the Panel in developing its recommendations on the validation status of these methods.

DATES: Nominations and data should be received by noon on May 5, 2005.

ADDRESSES: Nominations and data should be sent by mail, fax, or email to Dr. William S. Stokes, Director of NICEATM at NICEATM, NIEHS, P.O. Box 12233, MD EC–17, Research Triangle Park, NC, 27709, (phone) 919–541–2384, (fax) 919–541–0947, (e-mail) *niceatm@niehs.nih.gov*. Courier address: NICEATM, 79 T.W. Alexander Drive, Building 4401, Room 3128, Research Triangle Park, NC 27709.

FOR FURTHER INFORMATION CONTACT: Dr. William S. Stokes, Director of NICEATM, (phone) 919–541–2384, (fax) 919–541–0947, (email) *niceatm@niehs.nih.gov.*

SUPPLEMENTARY INFORMATION:

Background

In June 2004, the Environmental Protection Agency (EPA) asked ICCVAM to evaluate the validation status of proposed non-animal approaches for determining the skin and eye irritation potential of antimicrobial cleaning product formulations for meeting regulatory hazard classification and labeling requirements. ICCVAM considered the EPA's request and recommended that the evaluation of these non-animal approaches proceed as a high priority. ICCVAM agreed to work with the EPA and representatives of its Pesticide Program Dialogue Committee (PPDC) to help assure that the submission provided to ICCVAM contains all relevant information, data, and appropriate analyses as described in the ''ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods'' (NIH publication 03–4508). The NICEATM on behalf of ICCVAM plans to convene an independent scientific expert panel to review the submission, develop conclusions on the validation status of these methods, and make recommendations about the usefulness and limitations of these methods for their intended purpose. The date for the expert panel meeting has not been determined but will be announced in a future **Federal Register** notice.

Request for Data

Data, the nomination of experts, and other information submitted in response to this notice should be sent to NICEATM at the address given above. Data received by the deadline will be made available on the ICCVAM/NICEATM Web site at *http://iccvam.niehs.nih.gov* and considered by the Panel and ICCVAM.

When submitting data or information on protocols, please reference this **Federal Register** notice and provide appropriate contact information (name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization, as applicable). NICEATM prefers the submission of raw untransformed data in addition to any summary data including the submission of copies of pages from applicable study notebooks and/or study reports, if available. *In vivo* and *in vitro* data for each substance are preferred. Post-marketing surveillance data, ethical human studies, and accidental exposure reports also are sought when available and applicable. Each submission for a chemical or product should preferably include the following information when available:

- Common and trade name.
- Chemical Abstracts Service Registry Number (CASRN) for each ingredient of a formulation, and the percent composition of each ingredient.
- Chemical structure.
- Chemical class.
- Product class.
- Commercial source.
- Test protocol used for either *in vivo* or *in vitro* testing.
- The extent to which the study complies with national/international Good Laboratory Practice (GLP) guidelines.
- Date and testing organization.

Request for the Nomination of Scientists for the Expert Panel

NICEATM invites the nomination of scientists with relevant knowledge and experience that can serve on the Panel to evaluate *in vitro* dermal and ocular toxicity test methods. Areas of relevant expertise include, but are not limited to: human and animal dermatotoxicology/ophthalmology with an emphasis on evaluation and treatment of chemical injuries, *in vivo* dermal/ocular toxicity testing, *in vitro* dermal/ocular toxicology, test method validation, and biostatistics. Each nomination should include the person's name, affiliation, contact information (*i.e.*, mailing address, e-mail address, telephone and fax numbers), a brief summary of relevant experience and qualifications, and curriculum vitae, if possible. NICEATM and ICCVAM will also consider nominations previously submitted in response to a request for scientific experts for the evaluation of *in vitro* ocular test methods (**Federal Register**, Vol. 69, No. 57, pp. 13859–13861, March 24, 2004, available at *http://iccvam.niehs.nih.gov/)* and do not need to be resubmitted.

Background Information on ICCVAM and NICEATM

ICCVAM is an interagency committee composed of representatives from 15 Federal regulatory and research agencies that use or generate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability and promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety and hazards of chemicals and products and that refine, reduce, or replace animal use. The ICCVAM Authorization Act of 2000 (Pub. L. 106–545, available at *http://iccvam.niehs.nih.gov/about/*

PL106545.htm) establishes ICCVAM as a permanent interagency committee of the NIEHS under the NICEATM. NICEATM administers the ICCVAM and provides scientific and operational support for ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of Federal agencies. Additional information about ICCVAM and NICEATM can be found at the following Web site: *http://iccvam.niehs.nih.gov.*

Dated: March 9, 2005.

Samuel Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 05–5471 Filed 3–18–05; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Toxicology Program (NTP), NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); Request for Data on the Use of Topical Anesthetics and Systemic Analgesics for In Vivo Eye Irritation Testing

AGENCY: National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH).

ACTION: Request for data on the use of topical anesthetics and systemic analgesics for in vivo ocular irritation testing.

SUMMARY: The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and NICEATM request the submission of data and information on the use of topical anesthetics and systemic analgesics for alleviating pain and distress in rabbits during eye irritation testing. They also request the submission of information about other procedures and strategies that may reduce or eliminate pain and distress associated with *in vivo* eye irritation methods.

DATES: Data should be received by June 25, 2007.

ADDRESSES: Data should be sent by mail, fax, or e-mail to Dr. William S. Stokes, Director, NICEATM, NIEHS, P.O. Box 12233, MD EC–17, Research Triangle Park, NC, 27709, (fax) 919–541–0947, (e-mail) *niceatm@niehs.nih.gov. Courier address:* NICEATM, 79 T.W. Alexander Drive, Building 4401, Room 3128, Research Triangle Park, NC 27709.

FOR FURTHER INFORMATION CONTACT: Dr. William S. Stokes, NICEATM Director, (phone) 919–541–2384 or *niceatm@niehs.nih.gov*.

SUPPLEMENTARY INFORMATION

Background

The U.S. Environmental Protection Agency (EPA) nominated to ICCVAM several activities relevant to reducing, replacing, or refining the use of rabbits in the current *in vivo* eye irritation test method (**Federal Register** Vol. 69, No. 57, pages 13859–13861, March 24, 2004). One activity is to review ways to alleviate pain and suffering that might arise from current *in vivo* eye irritation testing. ICCVAM endorsed this activity with a high priority and recommended that NICEATM review the data currently available on the use of topical anesthetics and/or systemic analgesics to reduce animal pain and distress.

As part of this review, NICEATM requests the submission of data from completed studies on the use of topical anesthetics and/or systemic analgesics for *in vivo* ocular irritancy testing. These data will be used to evaluate the validation status of the use of topical anesthetics and/or analgesics to reduce pain and distress for *in vivo* testing situations. ICCVAM and NICEATM also request the submission of information and data from *in vivo* methods, procedures, and/or strategies that may reduce or eliminate the pain and suffering associated with current *in vivo* eye irritation methods.

Background Information on ICCVAM and NICEATM

ICCVAM is an interagency committee composed of representatives from 15 federal regulatory and research agencies that use or generate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability and promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety and hazards of chemicals and products and that refine, reduce, or replace animal use. The ICCVAM Authorization Act of 2000 (42 U.S.C. 285*l*–3) established ICCVAM as a permanent interagency committee of the NIEHS under NICEATM. NICEATM administers the ICCVAM and provides scientific and operational support for ICCVAM-related activities. Additional information about NICEATM and ICCVAM can be found at the following Web site: *http://iccvam.niehs.nih.gov*.

Dated: April 30, 2007.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences and National Toxicology Program.

[FR Doc. E7–8898 Filed 5–8–07; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); Request for Ocular Irritancy Test Data From Human, Rabbit, and In Vitro Studies Using Standardized Testing Methods

AGENCY: National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH).

ACTION: Request for submission of relevant data.

SUMMARY: The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and NICEATM are collaborating with the European Centre for the Validation of Alternative Methods (ECVAM) to evaluate the validation status of *in vitro* test methods for assessing the ocular irritation potential of substances. On behalf of the ICCVAM, NICEATM requests data on substances tested for ocular irritancy in humans, rabbits, and/or in vitro. These data will be used to: (1) Review the state-of-the-science in regard to the availability of accurate and reliable *in vitro* test methods for assessing the range of potential ocular irritation activity, including whether ocular damage is reversible or not and (2) expand NICEATM's high-quality ocular toxicity database. *In vitro* test methods for which data are sought include, but are not limited to: (1) The Bovine Corneal Opacity and Permeability (BCOP) test, (2) the Isolated Rabbit Eye (IRE) test, (3) the Isolated Chicken Eye (ICE) test, and (4) the Hen's Egg Test—Chorioallantoic Membrane (HET–CAM).

DATES: Data should be received by July 23, 2007. Data received after this date will be considered as feasible.

ADDRESSES: Dr. William S. Stokes, NICEATM Director, NIEHS, P.O. Box 12233, MD EC–17, Research Triangle Park, NC 27709, (fax) 919–541–0947, (e-mail) *niceatm@niehs.nih.gov. Courier address:* NICEATM, 79 T.W. Alexander Drive, Building 4401, Room 3128, Research Triangle Park, NC 27709. Responses can be submitted electronically at the ICCVAM–NICEATM Web site: *http://iccvam.niehs.nih.gov/contact/FR_pubcomment.htm* or by e-mail, mail, or fax.

FOR FURTHER INFORMATION CONTACT: Other correspondence should be directed to Dr. William S. Stokes (919–541–2384 or *niceatm@niehs.nih.gov*).

SUPPLEMENTARY INFORMATION:

Background

In October 2003, the U.S. Environmental Protection Agency (EPA) submitted to ICCVAM a nomination with several activities related to reducing, replacing, and refining the use of rabbits in the current *in vivo* eye irritation test method (**Federal Register** Vol. 69, No. 57, pp 13859–13861, March 24, 2004). In response to this nomination, ICCVAM completed an evaluation of the validation status of the BCOP, ICE, IRE, and HET–CAM test methods for identifying severe (irreversible) ocular irritants/corrosives using the United Nations Globally

Harmonized System of Classification and Labeling of Chemicals (GHS), the EPA, and the European Union hazard classification systems. NICEATM and ICCVAM prepared a comprehensive background review document (BRD) on each of the four *in vitro* test methods. Each BRD included an analysis of test method performance (i.e., reliability and relevance) as compared to the *in vivo* rabbit eye reference test method, based on all available data. ICCVAM developed recommendations on the usefulness and limitations of these *in vitro* test methods for identifying ocular corrosives/severe irritants after considering the BRDs, comments received from the public and the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), and comments and recommendations received from an independent expert panel (**Federal Register** Vol. 70, No. 53, pp 13513–13514, March 21, 2005 and Vol. 70, No. 211, p 66451, November 2, 2005).

ICCVAM is now reviewing the validation status of these and other *in vitro* test methods for identifying nonsevere ocular irritants (i.e., those that induce reversible ocular damage) and non-irritants.

Request for Data

As part of the review process, NICEATM requests the submission of data from substances tested for ocular irritancy in humans, rabbits, and/or *in vitro*. Data received by July 23, 2007 will be compiled and added to the database maintained by NICEATM and utilized where appropriate in the evaluation of *in vitro* ocular irritation test methods. Data received after this date will also be considered and used where applicable for future evaluation activities. All information submitted in response to this notice will be made publicly available upon request to NICEATM.

When submitting substance and protocol information/test data, please reference this **Federal Register** notice and provide appropriate contact information (name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization, as applicable).

NICEATM prefers data to be submitted as copies of pages from study notebooks and/or study reports, if available. Raw data and analyses available in electronic format may also be submitted. Each submission for a substance should preferably include the following information, as appropriate:

- Common and trade name.
- Chemical Abstracts Service Registry Number (CASRN).
- Chemical and/or product class.
- Commercial source.
- *In vitro* test protocol used.
- Rabbit eye test protocol used.
- Human eye test protocol used.
- Individual animal/human or *in vitro* responses at each observation time (i.e., raw data).
- The extent to which the study complied with national/international Good Laboratory Practice (GLP) guidelines.
- Date and testing organization.

Additional information on the submission of data may be obtained at *http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox.htm.*

Background Information on ICCVAM and NICEATM

ICCVAM is an interagency committee composed of representatives from 15 federal regulatory and research agencies that use or generate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability and promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety and hazards of chemicals and products and that refine, reduce, or replace animal use. The ICCVAM Authorization Act of 2000 (42 U.S.C. 285l–3, available at *http://iccvam.niehs.nih.gov/docs/about_docs/PL106545.pdf*) established ICCVAM as a permanent interagency committee of the NIEHS under NICEATM. NICEATM administers the ICCVAM and provides scientific and operational support for ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of federal agencies. Additional information about ICCVAM and NICEATM is available on the following Web site: *http://iccvam.niehs.nih.gov.*

Dated: May 25, 2007.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences and National Toxicology Program.

[FR Doc. E7–10966 Filed 6–6–07; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Toxicology Program (NTP); NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); Non-Animal Methods and Approach for Evaluating Eye Irritation Potential for Antimicrobial Cleaning Products (AMCPs): Request for Nominations for an Independent Expert Panel and Submission of Relevant Data

AGENCY: National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH).

ACTION: Request nominations for an independent expert panel and submission of relevant data.

SUMMARY: At the request of the U.S. Environmental Protection Agency (EPA), the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is planning to assess the validation status of a proposed non-animal approach for evaluating the eye irritation potential of AMCPs that meets hazard classification and labeling requirements. On behalf of ICCVAM, NICEATM requests:

1. Nominations of expert scientists to serve as members of an independent peer review panel.

2. Submission of relevant data and information on AMCPs or related substances obtained from (1) human testing or experience including reports from accidental exposures, (2) rabbits using the standard eye test or the low volume eye test (LVET), and (3) *in vitro* test methods for assessing ocular irritation, such as the Bovine Corneal Opacity and Permeability (BCOP) test, the Cytosensor Microphysiometer (CM) test, and the EpiOcular test, and data supporting the accuracy and reproducibility of these methods.

DATES: Submit nominations and data by May 19, 2008. Data submitted after this date will be considered in the evaluation, if feasible.

ADDRESSES: Submit nominations and data to Dr. William S. Stokes, NICEATM Director, NIEHS, P.O. Box 12233, MD EC–17, Research Triangle Park, NC, 27709, (fax) 919–541–0947 (e-mail) *niceatm@niehs.nih.gov.* Courier address: NICEATM, 79 T.W. Alexander Drive, Building 4401, Room 3128, Research Triangle Park, NC, 27709. Responses can also be submitted electronically via the ICCVAM–NICEATM Web site (*http://iccvam.niehs.nih.gov/contact/FR_pubcomment.htm*).

FOR FURTHER INFORMATION CONTACT: Other correspondence should be directed to Dr. William S. Stokes (919–541–2384 or *niceatm@niehs.nih.gov*).

SUPPLEMENTARY INFORMATION:

Background

In June 2004, the EPA Office of Pesticide Programs informed NICEATM that they were developing, via a subgroup of the Pesticide Program Dialogue Committee, a non-animal assessment approach for evaluating eye irritation potential and labeling requirements for AMCPs. Subsequently, the EPA in collaboration with the Alternative Testing Working Group (ATWG) developed a non-animal approach for this limited group of products. The ATWG is comprised of seven consumer product companies (Clorox, Colgate Palmolive, Dial, EcoLabs, Johnson Diversey, Procter & Gamble, and SC Johnson). The Institute for *In Vitro* Sciences, Inc. (IIVS), which coordinated the EPA–ATWG collaboration, performed additional testing to complete parallel sets of *in vivo* and *in vitro* data, and prepared a background review document (BRD) describing the final approach. More information concerning this submission is available at: *http://iccvam.niehs.nih.gov/methods/ocutox/AMCP.htm.*

In January 2008, IIVS submitted the BRD, *An In Vitro Approach for EPA Toxicity Labeling of Anti-Microbial Cleaning Products,* to NICEATM. The EPA and the ATWG requested that NICEATM and ICCVAM use information within the BRD to conduct a technical review of the proposed approach to determine whether ICCVAM could assure the EPA, with a reasonable degree of certainty, that the approach would be useful for making labeling decisions for AMCPs that appropriately inform the user.

NICEATM and ICCVAM are now conducting a preliminary evaluation of the submission to determine its completeness and adherence to ICCVAM guidelines, which are available at *http://iccvam.niehs.nih.gov/SuppDocs/SubGuidelines/SD_subg034508.pdf.* If they decide to move forward with an evaluation, NICEATM and ICCVAM will convene an independent peer review panel to review the validation status of the proposed approach.

Request for Nominations of Scientific Experts

NICEATM requests nominations of scientists with relevant knowledge and experience to serve on the peer review panel should it be convened. Areas of relevant expertise include, but are not limited to:

- Biostatistics
- Human and veterinary ophthalmology, with an emphasis on evaluation and treatment of chemical injuries
- *In vivo* ocular toxicity testing
- *In vitro* ocular toxicology
- Test method validation

Each nomination should include the nominee's name, affiliation, contact information (i.e., mailing address, e-mail address, telephone and fax numbers), curriculum vitae, and a brief summary of relevant experience and qualifications. Nominations previously submitted to NICEATM in response to an earlier request for scientific experts for a possible peer panel review of *in vitro* ocular test methods used to evaluate AMCPs (**Federal Register** Vol. 70, No. 53, pp. 13512–13513, available at *http://iccvam.niehs.nih.gov*) do not need to be resubmitted.

Request for Data

NICEATM invites the submission of relevant data and information on AMCPs or related substances obtained from (1) human testing or experience including reports from accidental exposures, (2) rabbits using the standard eye test or the low volume eye test (LVET), and (3) *in vitro* test methods for assessing ocular irritation, such as the Bovine Corneal Opacity and Permeability (BCOP) test, the Cytosensor Microphysiometer (CM) test, and the EpiOcular test, including data supporting the accuracy and reproducibility of these methods.

Although data can be accepted at any time, data received by May 19, 2008 will be considered during the ICCVAM

evaluation process. Relevant data received after this date will be considered during the ICCVAM evaluation process, if feasible. All information submitted in response to this notice will be made publicly available and may be incorporated into future NICEATM and ICCVAM reports and publications as appropriate.

When submitting data, please reference this **Federal Register** notice and provide appropriate contact information (name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization, as applicable).

NICEATM prefers that data be submitted as copies of pages from study notebooks and/or study reports, if available. Raw data and analyses available in electronic format may also be submitted. Each submission for a substance should preferably include the following information, as appropriate:

- Common and trade name
- Chemical Abstracts Service Registry Number (CASRN)
- Chemical and/or product class
- Commercial source
- *In vivo* or *in vitro* test protocol used
- Individual animal or *in vitro* responses at each observation time (i.e., raw data)
- The extent to which the study complied with national/international Good Laboratory Practice (GLP) guidelines
- Date and testing organization
- Physical and chemical properties (e.g. molecular weight, pH, water solubility, etc.)

Background Information on ICCVAM and NICEATM

ICCVAM is an interagency committee composed of representatives from 15 Federal regulatory and research agencies that use or generate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability and promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety and hazards of chemicals and products and that refine, reduce, and replace animal use. The ICCVAM Authorization Act of 2000 (42 U.S.C. 2851–3, available at (*http://iccvam.niehs.nih.gov/docs/about_docs/PL106545.pdf*) established ICCVAM as a permanent interagency committee of the NIEHS under NICEATM. NICEATM administers ICCVAM and provides scientific and operational support for ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of Federal agencies. Additional information about ICCVAM and NICEATM is available on the following Web site: *http://iccvam.niehs.nih.gov.*

Dated: March 24, 2008.

Samuel H. Wilson,

Acting Director, National Institute of Environmental Health Sciences and National Toxicology Program.

[FR Doc. E8–6969 Filed 4–3–08; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Toxicology Program (NTP); NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); Announcement of an Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods; Availability of Draft Background Review Documents (BRD); Request for Comments

AGENCY: National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH).

ACTION: Meeting announcement and request for comments.

SUMMARY: NICEATM, in collaboration with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), announces a public meeting of an independent scientific peer review panel (Panel) on alternative ocular safety testing methods. The Panel will evaluate (1) the validation status of a testing strategy that proposes the use of three *in vitro* test methods to assess the eye irritation potential of antimicrobial cleaning products (AMCPs), (2) the validation status of four *in vitro* test methods for identifying moderate (EPA Category II, UN Globally Harmonized System of Classification and Labeling of Chemicals (GHS) Category 2A) and mild (EPA Category III, GHS Category 2B) ocular irritants and substances not classified as ocular irritants (EPA Category IV, GHS Not Classified), (3) the validation status of the *in vivo* Low Volume Eye Test, and (4) a proposal for the routine use of topical anesthetics, systemic analgesics, and humane endpoints to avoid and minimize pain and distress during *in vivo* ocular irritation testing.

The Panel will review draft ICCVAM summary review documents and draft BRDs and evaluate the extent to which established validation and acceptance criteria have been adequately addressed for each proposed test method and strategy. The Panel also will be asked to comment on the extent to which the information included in the BRDs supports ICCVAM's draft test method recommendations.

NICEATM invites public comments on the draft ICCVAM summary review documents, BRDs, and draft ICCVAM test method recommendations. All documents will be available on the NICEATM–ICCVAM Web site at *http://iccvam.niehs.nih.gov/methods/ocutox/PeerPanel09.htm*. Documents will be posted no later than April 1, 2009.

DATES: The meeting is scheduled for May 19–21, 2009, from 8:30 a.m. to 5 p.m. each day. The deadline for registration to attend the meeting and submission of written comments is May 15, 2009.

ADDRESSES: The meeting will be held at the U.S. Consumer Products Safety Commission (CPSC) Headquarters, Bethesda Towers Building, 4330 East West Highway, Bethesda, MD. Persons needing special assistance in order to attend, such as sign language interpretation or other reasonable accommodation, should contact 301–402–8180 (voice) or 301–435–1908 TTY (text telephone) at least seven business days before the event.

FOR FURTHER INFORMATION CONTACT: Dr. William S. Stokes, Director, NICEATM, NIEHS, P.O. Box 12233, Mail Stop: K2–16, Research Triangle Park, NC 27709; (telephone) 919–541–2384; (fax) 919–541–0947; (e-mail) *niceatm@niehs.nih.gov*. Courier address: NICEATM, NIEHS, 530 Davis Drive, Room 2035, Durham, NC 27713.

SUPPLEMENTARY INFORMATION:

Background

In January 2008, a BRD titled *An In Vitro Approach for EPA Labeling of Anti-Microbial Cleaning Products* was submitted to NICEATM for review. This BRD, prepared by the Institute for *In Vitro* Sciences in collaboration with the Alternative Testing Working Group (comprised of seven consumer product companies [Clorox, Colgate Palmolive, Dial, EcoLabs, Johnson Diversey, Procter and Gamble, and SC Johnson]), describes a testing strategy that uses the Cytosensor Microphysiometer ®, EpiOcular ™, and Bovine Corneal Opacity and Permeability (BCOP) assays to assess the eye irritation potential of AMCPs and to determine the appropriate EPA ocular hazard classification category. NICEATM and ICCVAM reviewed the BRD, requested additional data and information, and compiled draft recommendations and a draft ICCVAM summary review document. The Panel will first consider the current validation status of each of the three *in vitro* test methods and then consider the validation status of the proposed testing strategy. The Panel will also review the validation status of the *in vivo* Low Volume Eye Test, which is proposed as reference data to partially substantiate the validity of the *in vitro* test methods used in the test strategy.

ICCVAM previously published recommendations on the use of four *in vitro* test methods (the BCOP, the isolated chicken eye test method, the isolated rabbit eye test method, and the hen's egg test-choriallantoic membrane test method) for identifying ocular corrosives and severe irritants for hazard classification and labeling purposes (available at *http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_tmer.htm*). The ICCVAM recommendations were submitted to and accepted by ICCVAM member agencies (*http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_recommend.htm*). One of the ICCVAM recommendations was to consider the validation status of these four *in vitro* ocular test methods for identifying mild and moderate ocular irritants and substances not classified as ocular irritants. NICEATM and ICCVAM have prepared draft BRDs assessing their current validation status for this purpose/application.

ICCVAM developed draft recommendations for the routine use of topical anesthetics, systemic analgesics, and humane endpoints to avoid or minimize pain and distress during *in vivo* ocular irritation testing. The proposal is based on recommendations by experts at a 2005 symposium *Minimizing Pain and Distress in Ocular Toxicity Testing* (co-sponsored by NICEATM–ICCVAM, the European Centre for the Evaluation of Alternative Methods [ECVAM], and the European Cosmetics Association) [*http://iccvam.niehs.nih.gov/meetings/ocumeet/sympinfo.htm*] that topical anesthetics and systemic analgesics should routinely be administered before ocular testing to avoid or minimize pain and distress that might occur during and after the initial application of test articles. The symposium experts also recommended that systemic analgesics should routinely be administered when there is evidence of potentially painful ocular damage or when there are clinical signs indicative of pain or distress. The experts also identified specific ocular injuries that would not be expected to reverse within 21 days, and therefore could be used as humane endpoints to end a study early. ICCVAM requested data (72 FR 26396) and then compiled available information on using topical anesthetics or systemic analgesics. The Panel will review the available information and comment on draft ICCVAM recommendations for the routine use of analgesics, anesthetics, and humane endpoints.

ICCVAM is also cooperating with ECVAM on the peer review evaluation of four cell-based *in vitro* ocular test methods by an ECVAM Scientific Advisory Committee (ESAC) Peer Review Panel. The four methods, Cytosensor ®, Fluorescein Leakage, Neutral Red Release, and the Red Blood

Haemaolysis Test Method, are being evaluated for their usefulness and limitations for identifying ocular corrosives and severe irritants (*i.e.*, EPA Category I, European Union (EU) R41, GHS Category 1) and substances not classified as ocular irritants (*i.e.*, EPA Category IV, EU Not Labeled, GHS Not Classified). ECVAM prepared BRDs for the four methods and links to these documents will be available on the ICCVAM Web site by April 1, 2009. ICCVAM developed draft recommendations on the usefulness and limitations of the four test methods based on the information in the BRDs. Public comments on the BRDs and draft recommendations are invited. The Panel will also be asked to comment on the ICCVAM draft recommendations.

Peer Review Panel Meeting

This meeting will take place May 19–21, 2009, at the CPSC Headquarters, Bethesda Towers Building, 4330 East West Highway, Bethesda, MD. It will begin at 8:30 a.m. and is scheduled to conclude each day at approximately 5 p.m. The meeting is open to the public at no charge, with attendance limited only by the space available. The Panel will consider the draft ICCVAM summary review documents and/or BRDs for each test method and evaluate the extent to which established validation and acceptance criteria are adequately addressed (as described in *Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods*, NIH Publication No. 97–3981, available at *http://iccvam.niehs.nih.gov/docs/about_docs/validate.pdf*). The Panel will then comment on the extent to which each of the draft ICCVAM test method recommendations is supported by the information provided in the corresponding draft BRD(s). The Panel is expected to review the test methods and testing strategy for labeling AMCPs first, followed by the four test methods used to identify mild and moderate irritants, and finally the use of anesthetics, analgesics, and humane endpoints when conducting *in vivo* eye irritation tests in rabbits.

Additional information about the meeting, including a roster of the Panel members and the draft agenda, will be posted on the NICEATM–ICCVAM Web site (*http://iccvam.niehs.nih.gov/methods/ocutox/PeerPanel09.htm*) two weeks before the meeting. This information will also be available after that date by contacting NICEATM (see **FOR FURTHER INFORMATION CONTACT** above).

Attendance and Registration

In order to facilitate planning for this meeting, persons wishing to attend are asked to register by May 15, 2009, via the NICEATM–ICCVAM Web site (*http://iccvam.niehs.nih.gov/contact/reg_form_OcuPanel.htm*). Visitor information, area map, driving directions, and CPSC contact information are available at *http://www.cpsc.gov/about/contact.html*.

Availability of the Documents

The draft summary review documents, draft BRDs, and draft ICCVAM test method recommendations will be posted no later than April 1, 2009, on the NICEATM–ICCVAM Web site (*http://iccvam.niehs.nih.gov/methods/ocutox/PeerPanel09.htm*), or by contacting NICEATM (see **FOR FURTHER INFORMATION CONTACT** above).

Request for Public Comments

NICEATM invites the submission of written comments on the draft ICCVAM summary review documents, draft BRDs, and draft ICCVAM test method recommendations by May 15, 2009. NICEATM prefers that comments be submitted electronically via the NICEATM–ICCVAM Web site (*http://iccvam.niehs.nih.gov/contact/FR_pubcomment.htm*) or via e-mail to *niceatm@niehs.nih.gov*. Written comments may also be sent by mail, fax, or email to Dr. William Stokes, Director, NICEATM, at the address listed above (see **FOR FURTHER INFORMATION CONTACT**). When submitting written comments, please refer to this **Federal Register** notice and include appropriate contact information (name, affiliation, mailing address, phone, fax, email, and sponsoring organization, if applicable). NICEATM will post all comments on the NICEATM–ICCVAM Web site (*http://iccvam.niehs.nih.gov*) identified by the individual's name and affiliation or sponsoring organization (if applicable). NICEATM will provide these comments to the Panel and ICCVAM agency representatives and make them available to the public at the meeting.

Opportunity will be provided for members of the public to present oral comments at designated times during the peer review. Up to seven minutes will be allotted per speaker. If you wish to present oral statements at the meeting (one speaker per organization), contact NICEATM (see **FOR FURTHER INFORMATION CONTACT** above) by May 15, 2009. Please provide a written copy of your comments with contact information (name, affiliation, mailing address, phone, fax, email, and sponsoring organization, if applicable) when registering to make oral comments. If it is not possible to provide a copy of your statement in advance, please bring 40 copies to the meeting for distribution to the Panel and to supplement the record. Written statements can supplement and expand the oral presentation. Please provide NICEATM with copies of any supplementary written statement using the guidelines outlined above.

Summary minutes and the Panel's final report will be available following the meeting on the NICEATM–ICCVAM Web site (*http://iccvam.niehs.nih.gov*). ICCVAM will consider the Panel's conclusions and recommendations and any public comments received in finalizing their test method recommendations for these methods.

Background Information on ICCVAM and NICEATM

ICCVAM is an interagency committee composed of representatives from 15 Federal regulatory and research agencies that use, generate, or disseminate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability and promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety and hazards of chemicals and products and that refine, reduce, and replace animal use. The ICCVAM Authorization Act of 2000 (42 U.S.C. 285l–3) established ICCVAM as a permanent interagency committee of the NIEHS under NICEATM. NICEATM administers ICCVAM and provides scientific and operational support for ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of U.S. Federal agencies. Additional information about ICCVAM and NICEATM can be found on their Web site (*http://iccvam.niehs.nih.gov*).

Dated: March 20, 2009.

John R. Bucher,

Associate Director, NTP.

[FR Doc. E9–7220 Filed 3–30–09; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Toxicology Program (NTP); Office of Liaison, Policy and Review; Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

AGENCY: National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH).

ACTION: Meeting announcement and request for comments.

SUMMARY: Pursuant to section 10(a) of the Federal Advisory Committee Act, as amended (5 U.S.C. Appendix 2), notice is hereby given of a meeting of SACATM on June 25–26, 2009, at the Hilton Arlington Hotel, 950 North Stafford Street, Arlington, VA 22203. The meeting is open to the public with attendance limited only by the space available. SACATM advises the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), and the Director of the NIEHS and NTP regarding statutorily mandated duties of ICCVAM and activities of NICEATM.

DATES: The SACATM meeting will be held on June 25 and 26, 2009. The meeting is scheduled from 8:30 a.m. to 5 p.m. on June 25 and 8:30 a.m. until adjournment on June 26, 2009. All individuals who plan to attend are encouraged to register online at the NTP Web site (*http://ntp.niehs.nih.gov/go/7441*) by June 17, 2009. In order to facilitate planning, persons wishing to make an oral presentation are asked to notify Dr. Lori White, NTP Executive Secretary, via online registration, phone, or e-mail by June 17, 2009 (see **ADDRESSES** below). Written comments should also be received by June 17, 2009, to enable review by SACATM and NIEHS/NTP staff before the meeting.

ADDRESSES: The SACATM meeting will be held at the Hilton Arlington Hotel, 950 North Stafford Street, Arlington, VA 22203 [hotel: (703) 528–6000)]. Public comments and other correspondence should be directed to Dr. Lori White (NTP Office of Liaison, Policy and Review, NIEHS, P.O. Box 12233, MD K2–03, Research Triangle Park, NC 27709; telephone: 919–541–9834 or e-mail: *whiteld@niehs.nih.gov*). Courier address: NIEHS, 530 Davis Drive, Room 2136, Durham, NC 27713. Persons needing interpreting services in order to attend should contact 301–402–8180 (voice) or 301–435–1908 (TTY).

Requests should be made at least 7 days in advance of the meeting.

SUPPLEMENTARY INFORMATION:

Preliminary Agenda Topics and Availability of Meeting Materials

Preliminary agenda topics include:

- NICEATM–ICCVAM Update.
- Regulatory Acceptance of ICCVAM–Recommended Alternative Test Methods.
- NRC Report *Recognition and Alleviation of Pain in Laboratory Animals*.
- Implementation of NICEATM–ICCVAM Five-Year Plan.
- Federal Agency Research, Development, Translation, and Validation Activities Relevant to the NICEATM–ICCVAM Five-Year Plan (EPA and USDA).
- Report on second meeting of Independent Peer Review Panel: Evaluation of the Updated Validation Status of New Versions and Applications of the Murine Local Lymph Node Assay: Assessing the Allergic Contact Dermatitis Potential of Chemicals and Products.
- Report on the Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods.
- Update from the Japanese Center for the Validation of Alternative Methods.
- Update from the European Centre for the Evaluation of Alternative Methods.
- Update from Health Canada.

A copy of the preliminary agenda, committee roster, and additional information, when available, will be posted on the NTP Web site (*http://ntp.niehs.nih.gov/go/7441*) or available upon request (see **ADDRESSES** above). Following the SACATM meeting, summary minutes will be prepared and available on the NTP Web site or upon request.

Request for Comments

Both written and oral public input on the agenda topics is invited. Written comments received in response to this notice will be posted on the NTP Web site. Persons submitting written comments should include their name, affiliation (if applicable), and sponsoring organization (if any) with the document. Time is allotted during the meeting for presentation of oral comments and each organization is allowed one time slot per public comment period. At least 7 minutes will be allotted for each speaker, and if time permits, may be extended up to 10 minutes at the discretion of the chair. Registration for oral comments will also be available on-site, although time allowed for presentation by on-site registrants may be less than for pre-registered speakers and will be determined by the number of persons who register at the meeting.

Persons registering to make oral comments are asked to do so through the online registration form (*http://ntp.niehs.nih.gov/go/7441*) and to send a copy of their statement to Dr. White (see **ADDRESSES** above) by June 17, 2009, to enable review by SACATM, NICEATM–ICCVAM, and NIEHS/NTP staff prior to the meeting. Written statements can supplement and may expand the oral presentation. If registering on-site and reading from written text, please bring 40 copies of the statement for distribution and to supplement the record.

Background Information on ICCVAM, NICEATM, and SACATM

ICCVAM is an interagency committee composed of representatives from 15 Federal regulatory and research agencies that use, generate, or disseminate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability and promotes the development, scientific validation, regulatory acceptance, implementation, and national and international harmonization of new, revised, and alternative toxicological test methods that more accurately assess the safety and hazards of chemicals and products and that refine, reduce, and replace animal use. The ICCVAM Authorization Act of 2000 [42 U.S.C. 285*l*–3] established ICCVAM as a permanent interagency committee of the NIEHS under NICEATM. NICEATM administers ICCVAM and provides scientific and operational support for ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of U.S. Federal agencies. Additional information about ICCVAM and NICEATM can be found on their Web site (*http://iccvam.niehs.nih.gov*).

SACATM was established in response to the ICCVAM Authorization Act [Section 285*l*–3(d)] and is composed of scientists from the public and private sectors. SACATM advises ICCVAM, NICEATM, and the Director of the NIEHS and NTP regarding statutorily mandated duties of ICCVAM and activities of NICEATM. SACATM provides advice on priorities and activities related to the development, validation, scientific review, regulatory acceptance, implementation, and national and international harmonization of new, revised, and alternative toxicological test methods. Additional information about SACATM, including the charter, roster, and records of past meetings, can be found at *http://ntp.niehs.nih.gov/go/167*.

Dated: April 22, 2009.

John R. Bucher,

Associate Director, National Toxicology Program.

[FR Doc. E9–9845 Filed 4–28–09; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Toxicology Program (NTP); NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches: Notice of Availability and Request for Public Comments

AGENCY: National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH).

ACTION: Request for comments.

SUMMARY: NICEATM, in collaboration with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), convened an independent international scientific peer review panel (hereafter, Panel) on May 19–21, 2009, to evaluate test methods and approaches with the potential to reduce and refine the use of animals for ocular safety testing. These evaluations included the following:

- A proposal for the routine use of topical anesthetics, systemic analgesics, and humane endpoints to avoid and minimize pain and distress during in vivo ocular irritation testing.
- The in vivo low volume eye test (LVET).
- The use of the bovine corneal opacity and permeability (BCOP), the Cytosensor Microphysiometer® (CM), the isolated chicken eye (ICE), the isolated rabbit eye (IRE), and the hen's egg test—chorioallantoic membrane (HET–CAM) test methods for identifying moderate and mild ocular irritants and substances not labeled as ocular irritants.
- Nonanimal testing strategies that use the BCOP, CM, and/or EpiOcular™ (EO) test methods to assess the eye irritation potential of antimicrobial cleaning products to determine their appropriate U.S. Environmental Protection Agency ocular hazard classification.

The Panel report from this meeting is now available. The report contains (1) The Panel's evaluation of the validation status of the test methods and testing strategies and (2) the Panel's comments on the draft ICCVAM test method recommendations. NICEATM invites public comment on the Panel report. The report is available on the NICEATM–ICCVAM Web site at *http://iccvam.niehs.nih.gov/docs/ocutox_docs/OcularPRPRept2009.pdf* or by contacting NICEATM at the address given below.

DATES: Written comments on the Panel report should be received by August 28, 2009.

ADDRESSES: NICEATM prefers that comments be submitted electronically by e-mail to *niceatm@niehs.nih.gov.* Comments can also be submitted via the NICEATM–ICCVAM Web site at *http://iccvam.niehs.nih.gov/contact/FR_pubcomment.htm.* Written comments can be sent by mail or fax to Dr. William S. Stokes, Director, NICEATM, NIEHS, P.O. Box 12233, Mail Stop: K2–16, Research Triangle Park, NC 27709; (fax) 919–541–0947. Courier address: NIEHS, NICEATM, 530 Davis Drive, Room 2035, Durham, NC 27713.

FOR FURTHER INFORMATION CONTACT: Dr. William S. Stokes, (telephone) 919–541–2384, (fax) 919–541–0947 and (e-mail) *niceatm@niehs.nih.gov.*

SUPPLEMENTARY INFORMATION:

Background

NICEATM announced the convening of an independent scientific peer review

panel to review and comment on the draft background review documents (BRDs) and summary review documents (SRDs) and draft recommendations, as well as the availability of the draft documents for public comment, in March 2009 (74 FR 14556). The Panel met in public session on May 19–21, 2009, at Consumer Product Safety Commission Headquarters in Bethesda, MD. The Panel reviewed the draft ICCVAM documents for completeness, errors, and omissions of any existing relevant data or information. The Panel then evaluated the information in the draft documents to determine the extent to which each of the applicable criteria for validation and acceptance of toxicological test methods (ICCVAM 2003) had been appropriately addressed. The Panel then considered the ICCVAM draft recommendations and commented on the extent that the recommendations were supported by the information provided in the draft BRDs or SRDs.

ICCVAM organized a 2005 symposium (70 FR 18037) on Minimizing Pain and Distress in Ocular Toxicity Testing where experts recommended that topical anesthetics and systemic analgesics should be routinely administered before in vivo ocular safety testing to avoid or minimize pain and distress that might occur during and after the initial application of test substances. The experts also recommended that systemic analgesics should routinely be administered when there are clinical signs indicative of pain or distress. The experts further recommended that humane endpoints to end a study early should be identified and used routinely. ICCVAM requested data (72 FR 26396), compiled available information on the use of topical anesthetics, systemic analgesics, and humane endpoints during in vivo ocular safety testing, and developed draft recommendations for implementing such practices.

In 2007, ICCVAM published (70 FR 66451) recommendations on the use of four in vitro test methods (BCOP, ICE, IRE, HET–CAM) for identifying ocular corrosives and severe irritants for hazard classification and labeling purposes. The ICCVAM recommendations were submitted to and accepted by ICCVAM member agencies (more information at *http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_recommend.htm*). One of the ICCVAM recommendations was to consider the validation status of these four in vitro ocular test methods for identifying mild and moderate ocular irritants and substances not classified as ocular irritants. NICEATM and ICCVAM requested data (72 FR 31582), compiled available information, prepared draft BRDs assessing their current validation status for this purpose, and developed draft recommendations for their use.

In January 2008, a BRD titled, An In Vitro Approach for EPA Labeling of Anti-Microbial Cleaning Products, was submitted to NICEATM for review. This BRD, prepared by the Institute for In Vitro Sciences in collaboration with the Alternative Testing Working Group (comprised of seven consumer product companies [Clorox, Colgate Palmolive, Dial, EcoLabs, Johnson Diversey, Procter and Gamble, and SC Johnson]), proposes a testing strategy that uses the CM®, EpiOcular™, and BCOP test methods to assess the eye irritation potential of antimicrobial cleaning products and to determine appropriate EPA ocular hazard classification categories for such products. NICEATM and ICCVAM reviewed the BRD, requested additional data and information (73 FR 18535), and compiled draft recommendations and a draft ICCVAM SRD. ICCVAM also reviewed the validation status of the LVET, which is proposed as a reference test method to partially substantiate the validity of the in vitro test methods used in the test strategy.

Availability of the Peer Panel Report

The Panel's conclusions and recommendations are detailed in the Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches which is available along with the draft documents reviewed by the Panel and the draft ICCVAM test method recommendations at *http://iccvam.niehs.nih.gov/methods/ocutox/PeerPanel09.htm.*

Request for Public Comments

NICEATM invites the submission of written comments on the Panel report. When submitting written comments, please refer to this **Federal Register** notice and include appropriate contact information (name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization, if applicable). All comments received will be made publicly available via the NICEATM–ICCVAM Web site at *http://iccvam.niehs.nih.gov/methods/ocutox/PeerPanel09.htm.* ICCVAM will consider the Panel report along with public comments and comments made by the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) at their June 25–26, 2009 meeting (74 FR 19562) when finalizing test method recommendations. Final ICCVAM recommendations will be published in ICCVAM test method evaluation reports, which will be forwarded to relevant Federal agencies for their consideration. The evaluation reports will also be available to the public on the NICEATM–ICCVAM Web site at *http://iccvam.niehs.nih.gov/methods/ocutox/ocutox.htm* and by request from NICEATM (see **ADDRESSES** above).

Background Information on ICCVAM, NICEATM, and SACATM

ICCVAM is an interagency committee composed of representatives from 15 Federal regulatory and research agencies that use, generate, or disseminate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability, and promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety and hazards of chemicals and products and that refine, reduce, and replace animal use. The ICCVAM Authorization Act of 2000 (42 U.S.C. 285l–3) established ICCVAM as a permanent interagency committee of the NIEHS under NICEATM. NICEATM administers ICCVAM and provides scientific and operational support for ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of U.S. Federal agencies. Additional information about ICCVAM and NICEATM can be found on their Web site (*http://iccvam.niehs.nih.gov).*

SACATM was established January 9, 2002, and is composed of scientists from the public and private sectors (67 FR 11358). SACATM provides advice to the Director of the NIEHS, ICCVAM, and NICEATM regarding the statutorily mandated duties of ICCVAM and activities of NICEATM. Additional information about SACATM, including the charter, roster, and records of past meetings, can be found at *http://ntp.niehs.nih.gov/* see ''Advisory Board & Committees'' (or directly at *http://ntp.niehs.nih.gov/go/167*).

Reference

ICCVAM. 2003. ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods. NIH Publication No. 03–4508. Research Triangle Park, NC: NIEHS. Available at: *http://iccvam.niehs.nih.gov.*

Dated: July 3, 2009.

John R. Bucher,

Associate Director, NTP.

[FR Doc. E9–16388 Filed 7–10–09; 8:45 am]

BILLING CODE 4140–01–P

This page intentionally left blank

Appendix E2

Public Comments Received in Response to *Federal Register* Notices

70 FR 13512 (March 21, 2005)
Request for Data on Non-Animal Methods and Approaches for Determining Skin and Eye Irritation Potential of Antimicrobial Cleaning Product Formulations; Request for Nominations for an Independent Expert Panel

- No responses received.

72 FR 26396 (May 9, 2007)
Request for Data on the Use of Topical Anesthetics and Systemic Analgesics for *In Vivo* Eye Irritation Testing

72 FR 31582 (June 7, 2007)
Request for Ocular Irritancy Test Data from Human, Rabbit, and *In Vitro* Studies Using Standardized Testing Methods

- No responses received.

73 FR 18535 (April 4, 2008)
Non-Animal Methods and Approach for Evaluating Eye Irritation Potential for Antimicrobial Cleaning Products (AMCPs): Request for Nominations for an Independent Expert Panel and Submission of Relevant Data

- No responses received.

74 FR 14556 (March 31, 2009)
Announcement of an Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods; Availability of Draft Background Review Documents (BRDs); Request for Comments

Subject: Federal Register Notice Vol 72, No. 89, May 9, 2007 (p 26396)
Date: Monday, June 25, 2007 1:48 PM
From: Robert Guest

Dear Dr Stokes,

Safepharm Laboratories Ltd. (SPL) supports the activity of ICCVAM-NICEATM to review ways to alleviate pain and suffering that might arise from current in vivo eye irritation testing. In response to the Federal Register Request for Data on the Use of Topical Anesthetics and Systemic Analgesics for In Vivo Eye Irritation Testing (Vol 72, No. 89, May 9, 2007), I would like to inform you that SPL has a policy of use of local anaesthetics to minimise pain and distress on administration of test substances in rabbit eye irritation studies. Whilst SPL is unable to provide data for review without the permission of its Sponsors, we are willing to provide further details of our current procedures for use of local anaesthetic. We also consider that there may be ways in which we could provide data from studies involving the use of local anaesthetic, without breach of confidentiality. Unfortunately the data is not yet in a form suitable for transmission but this can be arranged if required.

Please do not hesitate to contact me if this is of interest to ICCVAM-NICEATM.

Yours sincerely,

Mr Robert Guest
Head of Alternative and Acute Toxicology
Safepharm Laboratories Ltd.

BASF
The Chemical Company

May 13, 2009

Dr. William S. Stokes,
Director,
NICEATM,
NIEHS,
P.O. Box 12233,
Mail Stop: K2– 16,
Research Triangle Park, NC 27709

Dear Dr. Stokes,

BASF SE through BASF Corporation is pleased to provide comments on the draft ICCVAM test method recommendations for alternative methods to evaluate eye irritation. BASF SE has extensive experience validating one of these methods and comparing the results to current methods. In summary, we provide comments on the HET-CAM assay based on our retrospective analysis of HET-CAM results generated during in-house routine testing. We are not providing comment on the BCOP assay because is just being established in the Laboratory for Acute Toxicology and has not yet been evaluated. My colleague, Dr. Arnhild Schrage, will attend to provide additional comment at the meeting.

Regards,

Raymond M. David, Ph.D., DABT
Manager, Toxicology

BASF Corporation
100 Campus Drive
Florham Park N.J. 07932
Tel: (800) 526-1072
www.basf.com/usa

Helping Make Products Better™

BASF
The Chemical Company

Comments on the Draft BRD for the HET-CAM method

a. General comment:

There exist various protocols, endpoints and prediction models, especially for the HET-CAM method, making a comparison of different studies difficult. This observation is reflected in the ICCVAM report 2006[1] where information was collected on roughly 260 substances in 383 HET-CAM studies (Draft HET-CAM BRD, line 1118ff). So many substances were tested that for the analysis of one single HET-CAM protocol, only 25 % of all studies could be used because of the differences in protocols and endpoints. However, the results could be compared to *in vivo* data, using a specific analysis of one protocol with its specific endpoints and fewer substances, e.g only 63 substances from 4 publications for the IS(A) analysis method (Draft HET-CAM BRD, line 1112 ff). Therefore, as recommended by ICCVAM, we emphasize the importance of determining one specific protocol and specific irritant endpoints.

b. Specific comments

- *Line 877-879: development of irritant endpoints (hemorrhage [bleeding], vascular lysis [blood vessel disintegration], and coagulation [intra-and extravascular protein denaturation]*
  In our hands, distinguishing between hemorrhage and lysis during microscopic observation is difficult, as both effects result in blood vessel leakage. We recommend either a detailed description of the observed effects within the protocol that helps to distinguish between both effects, or combine both effects in one endpoint, which would then be considered as part of the the calculation of the irritation score (line 897).

- *Line 975ff:* in vivo *data*
  In addition to the *in vivo* classification, including an *in vivo* score from the results of the rabbit eye studies would facilitate the comparison of *in vitro* and *in vivo* data, e.g. the MMAS = modified maximum average score used by Balls *et al.* (1995)[2].

- *Line 1162ff.: HET-CAM Test Method Accuracy*
  (1182-1186: overall or for specific chemical and physical classes)
  To improve the predictability of the HET-CAM method, we recommend an analysis after grouping the substances by their solubility in water or oil.
  Our retrospective analysis of 145 routinely tested substances (manuscript submitted to *Alternatives to Laboratory Animals* in April 2009)[3] revealed that the HET-CAM´s overall accuracy and the overall rates of false negatives or

[1] http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_brd_hetcam.htm
[2] Balls *et al.*, Toxic. In Vitro Vol. 9, No. 6, pp. 871-929, 1995).
[3] Schrage A, Gamer AO, van Ravenzwaay B, Landsiedel R. Experiences with the HET-CAM method in the routine testing of a broad variety of chemicals and formulations. Submitted.

BASF Corporation
100 Campus Drive
Florham Park N.J. 07932
Tel: (800) 526-1072

BASF
The Chemical Company

false positives made this assay inadequate. However, the HET-CAM was sufficiently specific (few false positives) for water soluble substances, and highly sensitive (no false negatives) for non-water- and oil-soluble substances. Therefore, the HET-CAM might be applicable for excluding severe ocular irritation among water-insoluble substances. A copy of the abstract is attached.

Abstract of the manuscript, submitted to ATLA in April 2009

Experiences with the HET-CAM method in the routine testing of a broad variety of chemicals and formulations

Arnhild Schrage, Armin O. Gamer, Bennard van Ravenzwaay, and Robert Landsiedel
BASF SE, Experimental Toxicology and Ecology, 67056 Ludwigshafen, Germany

Data on eye irritation are generally needed for hazard identification of chemicals. For the routine testing of a broad variety of chemicals and formulations we used the Hen´s Egg Test - chorioallantoic membrane (HET-CAM) method. In the course of a tiered testing strategy and due to the lack of regulatory acceptance we also performed the Rabbit Eye Irritation test according to the OECD Test Guideline 405.
76 % of the 145 tested substances were non to mild irritating and 13 % were identified as irritating *in vivo* according to the EU classification system (GHS: 61% or 28 %, respectively). The remaining 11 % were severe irritants *in vivo*, which was based on the irreversibility of effects and not due to sufficiently high irritation scores in the three days after application.
The retrospective analysis revealed, that the HET-CAM´s overall accuracy was 65 % and the overall rate of false negatives (FN) and false positives (FP) was 50 % or 33 %, respectively. The HET-CAM was sufficiently specific (few FP) for water solubles, but failed to identify nearly all severe irritants within this group. In contrast, it was highly sensitive (no FN) for non- and oil-soluble substances, but the specificity for this group was rather low.
Therefore, the HET-CAM is not useful in our tiered-testing strategy for eye irritation testing. But for water-insoluble substances it might be applicable in combination with another *in vitro* method, provided that the regulatory acceptance is given.

BASF Corporation
100 Campus Drive
Florham Park N.J. 07932
Tel: (800) 526-1072

May 5, 2009

William Stokes, D.V.M, D.A.C.L.A.M.
Director, NICEATM
National Toxicology Program
P.O. Box 12233, K2-16
Research Triangle, NC 27709

Dear Dr. Stokes:

This public comment is delivered in response to Federal Register Notice Volume 74, Number 60, pages 14556-14557. It addresses the Summary Review Document (SRD), "Draft ICCVAM Summary Review Document: The Low Volume Eye Test", April 1, 2009.

The Summary Review Document purports to address the suitability of LVET data as an in vivo reference against which in vitro data might be compared. The analysis is central to the evaluation of the Draft Summary Review Document: Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products Using *In Vitro* Alternative Test Methods. An SRD should demonstrate the high level of scholarship commensurate with its intended purpose. The completeness and veracity of the data presented and conclusions drawn are of interest to all of us working in the field of alternatives for the prediction of eye irritation in humans. A fundamental principle of scientific scholarship is the support of conclusion statements with data or reference to data. The reader may wish to review this SRD with that thought in mind. There are a number of key points in the SRD that might benefit from additional data and/or alternative interpretation. These points I should like to address in this public comment. The reader can then choose to include or ignore these additions as she or he feels appropriate. The points in question are repeated in several sections of this SRD. I will not try to address each occurrence but cite one representative passage. Each point begins with the specific text from the SRD followed by the comments.

1. "**Accidental eye injury is the leading cause of visual impairment in the U.S. and many of these injuries occur due to contact with workplace and household chemicals. According to the National Institute of Occupational Safety and Health, each day about 2000 U.S. workers have a job-related eye injury that requires medical treatment. Even more eye injuries occur in the home, with 125,000 eye injuries a year caused by accidents involving common household products such as oven cleaner and bleach (source American Academy of Ophthalmology).**"[lines 319-325] Eye irritation, from mild through severe, is a concern in the home and workplace, in sports and in military training. The overall incidence of accident-induced visual impairment is the result of mechanical injury, thermal burns, and chemical exposure. McGwin and collaborators report that the vast majority of eye injury come from mechanical trauma (i.e., contusions/abrasions and foreign body)[1]. What is more important to this SRD is the frequency of moderate to severe chemical injuries to the eye. Wagoner[2] has reviewed a series of published reports and concluded that alkali injuries (including those from certain high alkali household products) and to a lesser degree acid injuries are the primary chemical injuries observed in people. It is of interest that

personal-care, surfactant-based cleaning products (laundry, dishwashing, and the like) and household bleaches are not mentioned. A summary table alkali and acids materials most associated with human eye injury is redrawn from this reference and is provided in Attachment 1.

2. "**The majority of available LVET data were generated with surfactant-based mixtures or products which produce only a mild ocular irritant response or no response**"[280-281]…"**there is no information on the performance of known human corrosives in the LVET**"[285-286]. It is expected that the developer of the LVET would focus on product types within its portfolio. Looking at the types of products used in the home, surfactant-based cleaning products are common and so assessment of their eye irritation potential would be important. However, the final statement is quite surprising given the available literature. The pioneering mechanistic studies of Maurer and Jester [3-6] were performed using individual chemicals that included 37% formaldehyde, 8% NaOH, undiluted parafluoranaline, and 10% hydrogen peroxide. Griffith et al (1980) [7] used a series of chemicals to compare several instillation volumes (10, 30, 100 µL). With all three instillation volumes, several chemicals (29% SLS, 10% Acetic Acid, Calcium Hydroxide, (100%), and 38% Formaldehyde) produced severe damage that did not reverse in 21 days. NaOH, Acetic acid, and Calcium hydroxide are on the table provided in Attachment 1.
3. "**Gettings et al (1996) evaluated 25 surfactant formulations and their hazard classifications by the EPA and GHS, and reported several incidences of under prediction of an ocular corrosive or severe irritant in the Draize rabbit eye test by the LVET method.**"[281-284] The Cosmetics, Toiletries, and Fragrance Association (CTFA) produced some of the most useful data sets for the analysis of both the Draize and LVET in vivo tests as well as a wide range of in vitro assays. The Phase III work focused on surfactant-based formulations. All studies used the same batches of test material. Gettings et al (1996) [8] reported the Draize scores while Gettings et al (1998) [9] reported the low volume eye test scores. In their analysis of each data set, the authors used the Kay and Calandra (1960) [10] categories to assign degrees of irritancy potential. In both cases, the highest category assigned was Moderate. The EPA and GHS analysis was performed by others. Unfortunately, the GHS analyses (distribution of GHS categories) in tables 4-2 [435] and 4-4 [454] are incorrectly calculated. Products HZI (Skin Cleaner), HZK (Bubble Bath), and HZS (Shower Gel) produced lesions in one of the six animals treated in each group that did not recover by 21 days. Thus, these three test materials would be considered severe in the GHS scoring system. These errors in the GHS tables in turn impact some of the associated text [439-447]. Eye irritation categories obtained from a single in vivo assay are sometimes treated as absolutes, almost inherent properties of the test material (rather than properties of the test and associated regulatory interpretations/classification). The 6-rabbit test can be broken down into 20 unique combinations of 3 rabbits to model the current regulatory test. This type of bootstrap analysis provides some insight into the potential irritation categories that might be obtained with the test material (Attachment 2).
4. **"…comparative human data from clinical studies and accidental exposures proposed to support its accuracy are largely with substances that are mild or non-irritating. Ethical considerations have limited the severity of substances that can be tested in human clinical studies. Such data provide little assurance to the regulatory**

agencies charged with protecting public health that the LVET can provide adequate protection from substances that may cause moderate or severe ocular injuries in humans."[lines 304-310], all of **Section 5.0 Performance of the LVET vs. the Draize Rabbit Eye Test Considering Human Study Data and Experiences**"[lines 458-493] and "**Accidental exposures are not generally considered to be a reliable source of the true ocular hazard potential since such exposures are likely immediately follows by flushing the eyes with large volumes of water.**"[lines 594-597] Both the Draize and LVET assays are intended to address eye irritation potentials from non-irritating through stages to severe. Laying the basis, through clinical trials, to show the LVET (or other assays) as an effective predictor of irritation in the milder end of the spectrum seems quite appropriate. I hope most readers would also agree that clinical trials with severe eye irritants are both unethical and largely unnecessary. As mentioned before, we have a large body of data on severe (vision impairing) damage from accidental chemical exposure. Thus it is very surprising see the use of such data so roundly criticized by the NICEATM. The statements regarding the appropriateness of using epidemiological data (accidental exposure) seemed to have originated from the NICEATM as they supported neither by data nor reference from the ophthalmic literature. Together, these sections propose that a test designed to identify the degree of irritation potential of a test material and thus mitigate the risks from its accidental to humans cannot be calibrated or verified based upon the decades of human use and accidental exposure. A single or even small number of accidental exposures might not provide a robust picture of the human irritation potential. However, the National Electronic Injury Surveillance System (NEISS) database contains hundreds of reports over a wide range of product/chemical classes. From Appendix 11 of the International Association for Soaps, Detergents and Maintenance Products extensive BRD (Appendix A of the SRD), the 1980 to 1991 data are available for several kinds of cleaning product categories. In all cases, the exposed individual was seen by an emergency department. Here are several examples: Laundry soaps and detergents (230 exposures and all evaluated/treated and released), Dishwashing liquids (90 exposures and all evaluated/treated and released), Fabric treatments (30 exposed and all evaluated/treated and released), General purpose household cleaners (664 exposed and all evaluated/treated and released) and Household Bleaches (often with other cleaning products) (961 exposed and all but 4 evaluated/treated and released [the final disposition of these 4 individuals was not available from the data presented]). These data from emergency departments do not address specific products but do provide a strong sense of the irritation character of product classes. The somewhat higher irritation potential of the bleaches is consistent with the results of Maurer et al (2001) [3] using the LVET with 13% sodium hypochlorite. In this study, recovery extended past 7 days making this concentration of bleach an EPA Category II. The point here is very simple. To dismiss the use of epidemiological data for eye irritation is to fly in the face of rational science and the considerable efforts to identify and characterize human risk (including those efforts of the Consumer Product Safety Commission). To ignore these data is to reduce the current and future assessment of eye irritation to a matter of dogma (an un-testable belief) rather than data (a testable hypothesis).

5. "**In contrast, there are no documented instances where a substance with a hazard category determined in the Draize eye test produced a more severe hazard category response in humans following accidental exposure or ethical human studies**."[lines

314-317] This statement (assertion) has appeared in NICEATM-derived BRDs since 2004 and has yet to ever be supported by data. In assessing the validation (and appropriateness for regulatory use) of new tests, both the sensitivity and specificity are evaluated. Acceptable predictive capacity is found in the ability to identify both positive and negative responses relative to the reference test (or species of interest). In the ICCVAM evaluation of the four in vitro methods for the prediction severe eye irritants, this point was reaffirmed. The SRD statement above might be substantiated at some point by data, but even so, it refers only to sensitivity and ignores the need for specificity. It is the matter of specificity that makes the data from Gettings et al (1996 and 1998) so important. All of us have direct experience using such consumer products. To see the likes of gel cleaner, shampoo, and facial cleaner placed in the same hazard category as concentrated hydrofluoric acid, formaldehyde, sulfur mustard, and sodium hydroxide gives one pause. Where is the specificity? One is reminded on the Aesop's fable of the Sheppard Boy and the Wolf (Attachment 3). Specificity is the key to credibility.

I thank you for the opportunity to make this public comment and ask that it be made available to the Expert Panel and general public before the 19-21 May 2009 meeting. I also look forward to attending the Peer Review Panel meeting.

Sincerely yours,

/s/

John W. Harbell, Ph.D.
16334 Sunset Valley Drive
Dallas, Texas, 75248
johnharbell@sbcglobal.net

References

1. McGwin, G., et al., *Trends in eye injury in the United States, 1992-2001.* Investigative Ophthalmology & Visual Sciences, 2006. **47**(2): p. 521-527.
2. Wagoner, M., *Chemical injury of the eye: current concepts in pathology and therapy.* Survey of Ophthalmology, 1997. **41**(4): p. 275-313.
3. Maurer, J.K., et al., *Pathology of ocular irritation with bleaching agents in the rabbit low-volume eye test.* Toxicol Pathol, 2001. **29**(3): p. 308-19.
4. Maurer, J.K., et al., *Pathology of ocular irritation with acetone, cyclohexanol, parafluoroaniline, and formaldehyde in the rabbit low-volume eye test.* Toxicol Pathol, 2001. **29**(2): p. 187-99.
5. Maurer, J.K., R.D. Parker, and J.V. Jester, *Extent of initial corneal injury as the mechanistic basis for ocular irritation: key findings and recommendations for the development of alternative assays.* Regulatory Toxicology and Pharmacology, 2002. **36**(1): p. 106-17.

6. Jester, J.V., *Extent of corneal injury as a biomarker for hazard assessment and the development of alternative models to the Draize rabbit eye test.* Cutaneous and Ocular Toxicology, 2006. **25**(1): p. 41-54.
7. Griffith, J.F., et al., *Dose-response studies with chemical irritants in the albino rabbit eye as a basis for selecting optimum testing conditions for predicting hazard to the human eye.* Toxicology and Applied Pharmacology, 1980. **55**(3): p. 501-13.
8. Gettings, S.D., et al., *The CTFA Evaluation of Alternatives Program: an evaluation of in vitro alternatives to the Draize primary eye irritation test. (Phase III) surfactant-based formulations.* Food and Chemical Toxicology, 1996. **34**(1): p. 79-117.
9. Gettings, S.D., et al., *A comparison of low volume, Draize and in vitro eye irritation test data. III. Surfactant-based formulations.* Food Chem Toxicol, 1998. **36**(3): p. 209-31.
10. Kay, J.H. and J.C. Calandra, *Interpretation of eye irritation tests.* Journal of the Society of Cosmetic Chemists, 1962. **12**: p. 281-289.

Attachment 1

Common Causes of Chemical Injury*

Class	Compound	Common Sources/Use	Comments
Alkali	Ammonia [NH_3]	Fertilizer	Combines with water to form NH_40H fumes
		Refrigerants	Very rapid penetration
		Cleaning agents (7% solution)	
	Lye [NaOH]	Drain cleaner	Penetrates almost as rapidly as ammonia
	Potassium hydroxide [KOH]	Caustic potash	Similar to that of lye
	Magnesium hydroxide [$Mg(OH)_2$]	Sparklers	Produces combined thermal and alkali injury
	Lime [$Ca(OH)_2$]	Plaster	Most common cause of chemical injury in the work place
		Mortar	Poor penetration
		Cement	Toxicity increased by retained particulate matter
		Whitewash	
Acids	Sulfuric acid [H_2SO_4]	Industrial cleaners	Combines with water to produce corneal thermal injury
		Battery acid	May be associated with foreign body or laceration from batter acid
	Sulfurous acid [H_2SO_3]	Formed from sulfur dioxide (SO_2) by combination with corneal water	Penetrates more easily than other acids
		Fruit and vegetable preservatives	
		Bleach	
		Refrigerants	
	Hydofluoric acid [HF]	Glass polishing	Penetrates easily
		Glass frosting	Produces severe injury
		Mineral refining	
		Gasoline alkylation	
		Silicone	

		production	
	Acetic acid [CH_3COOH]	Vinegar 4-10%	Mild injury with less than 10% contamination
		Essence of vinegar 80%	Severe injury with higher concentration
		Glacial acetic acid 90%	
	Chromic acid [Cr_2O_3]	Used in the chrome plating industry	Chromic exposure produces chromic conjunctivitis with brown discoloration
	Hydrochloric acid [HCl]	Used as a 31-38% solution	Severe injury only with high concentration and prolonged exposure

- Redrawn from Wagoner, 1997 [2]

Attachment 2

The Draize and LVET eye irritation determinations were performed on 6 rabbits per test material per method using coded samples and a random block design. The studies were GLP compliant. The results from the 6 rabbits in each test group can be distributed into 20 unique combinations of 3 rabbits. Three rabbits are now the standard for the Draize and LVET assays. One can then compare the irritation category for each of the 20 combinations. Below are shown the original 6-rabbit category and the distribution of the categories of the 20 3-rabbbit categories. Only the EPA categories are shown for simplicity. These data illustrate the potential for rather disparate predictions when only one or two animals fail to recover among the six treated (see for example the Draize results for HZD or LVET results for HZI).

Draize Test (Gettings et al, 1996)

Code	Name	6-rabbit category	Distribution of 3-rabbit categories				# not cleared[1]	Average days to clear[2]
			EPA Cat I	EPA Cat II	EPA Cat III	EPA Cat IV		
HZA	Shampoo 7	I	16	4	0	0	2	12.3
HZB*	Liquid Soap 1	III	0	0	20	0	0	4.0
HZC*	Shampoo 1	III	0	0	20	0	0	5.2
HZD*	Shampoo 5	III	0	0	20	0	0	3.7
HZE	Gel Cleaner	I	10	0	10	0	1	4.2
HZF	Baby Shampoo 2	I	16	4	0	0	2	10.5
HZG*	Shampoo 8	III	0	0	20	0	0	3.5
HZH	Eye Makeup remover	IV	0	0	0	20	0	1.0
HZI	Skin Cleaner	I	19	1	0	0	3	9.3
HZJ	Mild Shampoo	IV	0	0	0	20	0	1.3
HZK	Bubble bath	I	20	0	0	0	5	7.0
HZL	Foam Bath	I	19	0	1	0	3	7.0
HZM*	Shampoo 3	III	0	0	10	10	0	2.3
HZN*	Shampoo 6	III	0	0	20	0	0	2.8
HZP	Baby Shampoo 1	III	0	0	19	1	0	2.7
HZQ	Cleaning Gel	III	0	0	20	0	0	3.5
HZR*	Facial Cleansing Foam	I	10	0	10	0	1	5.2
HZS	Shower Gel	I	19	1	0	0	3	9.3
HZT	Polishing Scrub	IV	0	0	0	20	0	1.0
HZU*	Hand Soap	III	0	0	20	0	0	4.5
HZV*	Shampoo 4	III	0	0	20	0	0	3.7
HZW*	Liquid Soap 2	III	0	0	20	0	0	6.0
HZX	Shampoo 2	I	16	4	0	0	3	9.3
HZY	Shampoo AntiD	I	16	4	0	0	2	12.3
HZZ	Facial Cleaner	IV	0	0	0	20	0	1.0

*Diluted to 25% in water before testing

[1] Number of animals that did not recover by 21 days

[2] The average number of days to clear in those animals that did clear by 21 days

LVET (Gettings et al, 1998)

Code	Name	6-rabbit category	Distribution of 3-rabbit categories				# not cleared[1]	Average days to clear[2]
			EPA Cat I	EPA Cat II	EPA Cat III	EPA Cat IV		
HZA	Shampoo 7	III	0	0	20	0	0	2.0
HZB*	Liquid Soap 1	IV	0	0	0	20	0	0.0
HZC*	Shampoo 1	III	0	0	20	0	0	2.0
HZD*	Shampoo 5	III	0	0	16	4	0	0.8
HZE	Gel Cleaner	III	0	0	20	0	0	1.3
HZF	Baby Shampoo 2	III	0	0	20	0	0	2.8
HZG*	Shampoo 8	III	0	0	19	1	0	1.3
HZH	Eye Makeup remover	IV	0	0	0	20	0	0.0
HZI	Skin Cleaner	I	10	0	10	0	1	3.8
HZJ	Mild Shampoo	IV	0	0	0	20	0	0.0
HZK	Bubble bath	I	10	0	10	0	1	4.2
HZL	Foam Bath	III	0	0	20	0	0	3.5
HZM*	Shampoo 3	III	0	0	19	1	0	1.0
HZN*	Shampoo 6	III	0	0	16	4	0	0.7
HZP	Baby Shampoo 1	III	0	0	10	10	0	0.3
HZQ	Cleaning Gel	IV	0	0	0	20	0	0.0
HZR*	Facial Cleansing Foam	III	0	0	16	4	0	0.7
HZS	Shower Gel	I	10	0	10	0	1	5.4
HZT	Polishing Scrub	IV	0	0	0	20	0	0.0
HZU*	Hand Soap	III	0	0	16	4	0	0.8
HZV*	Shampoo 4	III	0	0	10	10	0	0.3
HZW*	Liquid Soap 2	III	0	0	20	0	0	2.3
HZX	Shampoo 2	III	0	0	20	0	0	4.2
HZY	Shampoo AntiD	II	0	10	10	0	0	6.2
HZZ	Facial Cleaner	IV	0	0	0	20	0	0.0

*Diluted to 25% in water before testing

[1] Number of animals that did not recover by 21 days

[2] The average number of days to clear in those animals that did clear by 21 days

Attachment 3

Many of us will remember the short fables of Aesop from childhood. Fables tend to be a bit dramatic with morals directed to the proper upbringing of children. Thus, the moral here should not be over interpreted to the subject at hand. The importance of this fable is to remind us of the importance of raising the alarm only for real danger least all alarms be ignored.

"The Boy Who Cried Wolf, also known as *The Shepherd Boy and the Wolf*, is a fable attributed to Aesop (210 in Perry's numbering system[1]). The protagonist of the fable is a bored shepherd boy who entertained himself by calling out "Wolf!". Nearby villagers who came to his rescue found that the alarms were false and that they had wasted their time. When the boy was actually confronted by a wolf, the villagers did not believe his cries for help and the wolf ate the flock (and in some versions the boy). The moral is stated at the end of the fable as:

Even when liars tell the truth, they are never believed. The liar will lie once, twice, and then perish when he tells the truth."

(From: http://en.wikipedia.org/wiki/The_Boy_Who_Cried_Wolf)

MatTek Corporation

May 15, 2009

MatTek Corporation response to Federal Register Notice (Vol. 74(60):14556, 2009): Request for public comment on the background review document (BRD), draft ICCVAM summary review document (SRD), and draft ICCVAM recommendations on an In Vitro Approach for EPA Toxicity Labeling of Anti-Microbial Cleaning Products.

We are pleased that the EpiOcular model was chosen as one of the test systems for development of ocular hazard assessment assays as described in the Background Review Document (BRD) of an In Vitro Approach for EPA Toxicity Labeling of Anti-Microbial Cleaning Products. We also very much appreciate the efforts expended by the authors and the Alternative Testing Working Group to conduct the studies and prepare the documents. However, after careful review of the data presented in the BRD, we have concerns that this document significantly understates the true potential utility of the EpiOcular assay in comparison to the BCOP assay for this important application. Specific detailed comments are presented below. We request that the ICCVAM and the Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods consider these comments and incorporate them into the Final ICCVAM SRD and recommendations on this topic.

1. Removal of Restriction on Testing of Oxidant Chemicals in the EpiOcular Assay.

The BRD proposes a scheme for testing of anti-microbial cleaning products in which products containing oxidant chemicals are automatically excluded from testing in the EpiOcular assay (BRD, p xxiv). The rationale given for this decision is that EpiOcular test results did not match well (i.e. EpiOcular predicted more severe irritation) compared to in vivo data obtained in the Low Volume Eye Test (LVET) (BRD, Section 6, p108). The same comparison of BCOP data to LVET data was not made.

When compared to in vivo Draize data, EpiOcular test results with oxidant chemicals were in 100% agreement (BRD, Section 6, p116). In contrast, oxidant chemicals are known to be problematic in the BCOP test, even in comparison to Draize data. Oxidants are often under-predicted by the BCOP assay, and require histological assessment for correct prediction (BRD, Section 6, p135). Furthermore, BCOP data for oxidant chemicals showed only 62% correct predictions when compared to Draize data, with 19% over-prediction and 19% under-prediction (BRD, Section 6, p128). Additionally, as noted in the Draft Proposed ICCVAM Test Method Recommendations: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches (April 1, 2009) (Section 2.0, p11):

- LVET under-predicts severe irritants compared to the Draize.
- There are insufficient data to evaluate the extent of (LVET) under-prediction relative to known human severe ocular irritants.
- There is an inconsistent relationship between LVET and Draize results (i.e., time-to-clear) for substances with available human data.
- Accordingly, ICCVAM proposes that the LVET has not been adequately validated and does not have adequate demonstrated performance (sensitivity and specificity) to serve as an acceptable reference test method against which to determine the validity of *in vitro* alternative test methods for hazard classification and labeling purposes.

200 Homer Avenue
Ashland MA 01721 *Web Site:* **www.mattek.com** *Email:* **information@mattek.com**
(508) 881-6771
Fax (508) 879-1532

Thus, the rationale presented in the BRD for excluding EpiOcular for use with oxidant chemicals is flawed and this restriction on the EpiOcular assay should be removed. In making their draft recommendations, ICCVAM considered EpiOcular data presented for all chemicals including oxidants in their determination of the usefulness the EpiOcular assay. However, they did not explicitly comment on the recommendation presented in the BRD to automatically exclude oxidants from being tested in the EpiOcular assay. Based on the data presented in the BRD in comparison to in vivo Draize data, we ask ICCVAM and the Independent Peer Review Panel to explicitly comment on the usefulness of the EpiOcular Assay with oxidant chemicals and the unwarranted recommendation presented in the BRD to exclude the EpiOcular assay from use in testing oxidant chemicals.

2. Correction and Clarification of Criteria for Use of the EpiOcular Assay and the Cytosensor Assay.

The ICCVAM Draft Summary Review Document (SRD): Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products Using *In Vitro* Alternative Test Methods (SRD) (April 1, 2009) misstates the scheme proposed in the BRD (p 6, Figure 1-1) with regard to the appropriate criteria for use of the EpiOcular assay and the Cytosensor assay. The SRD (p xxi) incorrectly interprets the BRD scheme as stating that, "Selection of the CM (Cytosensor Microphysiometer) or EO (EpiOcular) depends on water solubility of the test substance; water-soluble substances would be tested in the CM and water-insoluble substances would be tested in the EO to determine the final hazard classification." The scheme actually indicates that water soluble test agents can be tested in either assay (BRD p122), but water insoluble test agents are incompatible with the Cytosensor assay, and therefore can only be tested in the EpiOcular assay. Although this may be a moot point given that Cytosensor instrumentation and related supplies are no longer be available, we ask that this statement be corrected in the SRD and clarified in the BRD.

3. Removal of Restriction on Use of the EpiOcular Assay for Determination of Category I (Cat I) Chemicals.

The BRD implies that the BCOP assay is most useful for the severe categories and that EpiOcular should only be used for the milder categories (BRD p xxxi). Papers published by Stern et al. (Toxicology In Vitro, 12, 455-461, (1998)) and Jones et al (ATLA 29, 669-692, (2001)) are cited with regard to assertions that the EpiOcular assay functions particularly well at the mild end of the ocular irritation spectrum (BRD p121). While this is indeed true, this should NOT, however, be interpreted as indicating that the EpiOcular assay does not function well at the severe end of the ocular irritation spectrum as well. In fact, the Jones et al paper states that, "The EpiOcular assay showed the closest concordance between the in vivo results and the in vitro data from cell-based assays…" Likewise, the Stern at al paper found an overall high concordance between in vivo Draize data and in vitro EpiOcular data. In contrast, Jones et al also found that, "The BCOP assay was less sensitive than the IRE test in discriminating between formulations of different irritation potentials, and did not perform as well as the other assays in identifying mild formulations."

The BRD also states that the EpiOcular assay ***cannot*** distinguish between Cat I and Cat II chemicals (BRD p xxxi), and that the BCOP ***can*** effectively distinguish between EPA Cat I and II chemicals (BRD xxxi). The data presented in the BRD are inconsistent with these claims as well.

A summary of the BCOP and EpiOcular data are complied for reference in Tables I and II below. The data summarized in Table I show that the performance of the EpiOcular assay was superior to that of the BCOP assay at both ends of the ocular irritation spectrum. The EpiOcular assay produced 100% sensitivity and 88% predictivity for Cat I chemicals, while for the BCOP assay, sensitivity ranged from 84-92% and predictivity ranged from 77-87%. Thus, while both the BCOP and EpiOcular assays appear to be useful for determining Cat I chemicals, the EpiOcular assay clearly performed better than the BCOP. Therefore, exclusion of the EpiOcular assay for determination of Cat 1 chemicals is not justified by the data and this restriction on the EpiOcular assay should be removed from the finalized testing strategy proposed in the BRD (p xxiv).

Regarding Cat II chemicals, EpiOcular was only tested with 1 chemical, which it underpredicted as a Cat III. Therefore, while additional testing of Cat II materials in the EpiOcular assay is warranted, the currently available data do not provide any basis for stating that the EpiOcular test cannot distinguish between Cat I and Cat II chemicals. For Cat II chemicals, the BCOP assay provided only very low sensitivity (ranging from 20-60%) and predictivity (ranging from 17-38%). Furthermore, following the procedure recommended for the BCOP in the BRD, for chemicals testing preliminarily as Cat II, "they should be further assessed with a histopathological evaluation and given the final categorization of whichever determination (in vitro score or histological evaluation) is more severe," (BRD, p145). This procedure is expected to overpredict 80% of Cat II chemicals as Cat I (BRD, Table 6-50, p141). Thus, the BCOP cannot be regarded as a useful assay for predicting Cat II chemicals or distinguishing between Cat I and Cat II chemicals.

Data presented in the BRD do show, however, that the high solvent (HS) BCOP assay plus histology is effective for distinguishing between Cat I plus II and Cat III (BRD Table 6-50, p141). Thus, after first removing true Cat I chemicals in preliminary tests (e.g with the EpiOcular assay), the BCOP plus histology assay (if fully developed and approved) may be useful for distinguishing between Cat II and Cat III (see new testing strategy proposed in section 4 below).

4. Proposal for Improved Testing Strategy for Use of the EpiOcular Assay and the BCOP Assay for Determination of EPA Hazard Classification of Anti-Microbial Cleaning Products.

With unwarranted restrictions on testing of oxidant chemicals and use for determination of Cat I chemicals removed from the EpiOcular assay, the following testing strategy for determination of EPA hazard classification of anti-microbial cleaning products is most consistent with the data presented in the BRD. This strategy will represent the best solution for the EPA in terms of accuracy, time required for assay performance, cost and ease of use (Figure 1) (see also BRD p122 for similar scheme). According to the proposed strategy, chemicals should be tested first in the EpiOcular assay to determine Cat I, Cat II plus III and Cat IV classifications. These 3 classifications will provide the most important information (i.e. irreversible, reversible or minimal eye irritation potential). If Cat I or Cat IV are determined, no further testing is required. For chemicals testing as Cat II plus III, if a broad classification of reversible is acceptable to the manufacturer, no further testing is required. However, if a distinction between Cat II (reversible within 21 days) and Cat III (reversible within 7 days) is desired, further testing with the BCOP (with solvent concentration accounted for and histology assessment, when fully developed) can be performed to determine Cat II or Cat III (with this assay protocol, no Cat II chemicals are underpredicted as Cat III, BRD, Table 6-50, P141). Further testing and refinement of the EpiOcular assay may also ultimately allow separation of Cat II and Cat III chemicals.

The ICCVAM draft SRD conducted an analysis of the 28 chemicals with available Draize data that were tested in common between the EpiOcular Assay and the BCOP assay (SRD Table 2, p xxx, and Appendix G). Two approaches utilizing BCOP for testing Cat I chemicals and EpiOcular for testing Cat IV chemicals were evaluated. One approach involved testing of all chemicals in the BCOP first, removing chemicals determined to be Cat I, and re-testing the remaining chemicals in the EpiOcular assay to determine Cat IV chemicals. The alternate approach tested the chemicals in the EpiOcular assay first, and after removal of all Cat IV chemicals, re-testing of the remaining chemicals in the BCOP assay to determine Cat I chemicals. Both approaches correctly categorized 79% of the test chemicals, and were described as useful for determining Cat I and Cat IV chemicals in the ICCVAM draft recommendations.

However, analysis of data presented in SRD Appendix G show that when the BCOP assay was performed first, only 64% of all chemicals including 100% of Cat I, but 0% of Cat IV chemicals were correctly categorized by the BCOP assay. However, if the EpiOcular assay is performed first, the 79% concordance is immediately obtained, including 100% Cat I, 100% of Cat III and 44% of Cat IV chemicals with no under-prediction of the more severe categories. Conducting the BCOP assay after the EpiOcular assay would thus unnecessarily waste time and resources without any added benefit.

Therefore, based on the data presented in the SRD, Appendix G, we ask ICCVAM and the Independent Peer Review Panel to accord the same degree of "usefulness" to the EpiOcular assay as a stand-alone assay as is accorded to the tiered assays combining BCOP and EpiOcular in the Final SRD and Recommendation documents.

5. Summary and Final Comments

Based on the detailed comments presented above, we request that the ICCVAM and the Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods consider the following items and incorporate specific responses to them in the Final ICCVAM SRD and Recommendations on this topic.

- Removal of the restriction on testing of oxidants in the EpiOcular assay.
- Correction and clarification of criteria for use of the EpiOcular Assay and the Cytosensor Assay.
- Removal of the restriction on use of the EpiOcular Assay for determination of Cat I chemicals.
- Proposal for improved testing strategy for use of the EpiOcular Assay and the BCOP Assay for determination of EPA hazard classification of anti-microbial cleaning products.
- Explicit acknowledgment that the EpiOcular assay provided overall superior performance compared to the BCOP assay for the common chemical set tested and compared against Draize data. Furthermore, acknowledgement that combining the BCOP and EpiOcular assay did not provide any benefit to results obtained by the EpiOcular assay alone.
- In addition to the ICCVAM finding that a combination of BCOP and EO appear to be useful for determination of Cat I and Cat IV, the EpiOcular assay has the identical utility for determining these categories by itself as a stand alone method. The BCOP assay in contrast is only useful for determining Cat I as a stand alone assay.

MatTek Corporation values and appreciates its close working relationship with the institutions and companies involved in development of animal alternative methods such as the EPA hazard classification methods proposed here. We look forward to continuing this close cooperation in order to develop any new EpiOcular data that may be required to support full validation of the EpiOcular in vitro test method for determination of EPA Toxicity Labeling of Anti-Microbial Cleaning Products. However, it is

important that the concerns raised here are adequately addressed in order to insure that unjustified restrictions are not imposed on the EpiOcular Assay during future studies.

Table I. Summary of BCOP and EpiOcular performance compared to Draize data presented in the BRD of an In Vitro Approach for EPA Toxicity Labeling of Anti-Microbial Cleaning Products

	BCOP			EpiOcular	
	Assay Sensitivity (Assay Predictivity) (%)[6]				
	Std Protocol[1]	HS Protocol/WO Hist[2]	HS Protocol/W Hist[3]	W Oxidiants[4]	WO Oxidants[5]
Cat I	90 (87)	84 (84)	92 (77)	100 (88)	100 (86)
Cat II	60 (27)	60 (38)	20 (17)	0 (0)[7]	0 (0)[7]
Cat III	50 (25)	58 (25)	58 (/28)	75 (38)	75 (38)
Cat IV	0 (0)	0 (0)	0 (0)	44 (100)	38 (100)
Overall Correct Classification	54.5 %	49 %	51 %	76 %	72 %

[1]Standard BCOP protocol: BRD Table 6-40, P128.
[2]High solvent BCOP protocol without histology: BRD Table 6-44, P133.

[3]High solvent BCOP protocol with histology: BRD Table 6-50, P141.
[4]EpiOcular data compared to Draize data including oxidant chemicals: BRD Table 6-29, P116.
[5]EpiOcular data compared to Draize data not including oxidant chemicals: BRD Table 6-31, P118.
[6]Sensitivity is defined as the proportion of true positives that are correctly identified by the test and predictivity is defined as the proportion of total positive predictions that are correct.
[7]Based on only 1 Cat II chemical tested.

Table II. Summary of BCOP and EpiOcular performance compared to Draize data presented in the BRD of an In Vitro Approach for EPA Toxicity Labeling of Anti-Microbial Cleaning Products: Cat I vs. Cat II-III-IV.

	BCOP		EpiOcular	
	Assay Sensitivity[1]			
	Std Protocol[2]	HS Protocol/WO Hist[3]	HS Protocol/W Hist[4]	EpiOcular[5]
Cat I	27/30 (90%)	21/25 (84%)	23/25 (92%)	15/15 (100%)
Cat II-III-IV	30/36 (83%)	32/36 (88.9%)	29/36 (80.5%)	12/14 (85.7)

[1]Sensitivity is defined as the proportion of true positives that are correctly identified by the test

[2]Standard BCOP protocol: BRD Table 6-40, P128.
[3]High solvent BCOP protocol without histology: BRD Table 6-44, P133.
[4]High solvent BCOP protocol with histology: BRD Table 6-50, P141.
[5]EpiOcular data compared to Draize data including oxidant chemicals: BRD Table 6-29, P116.

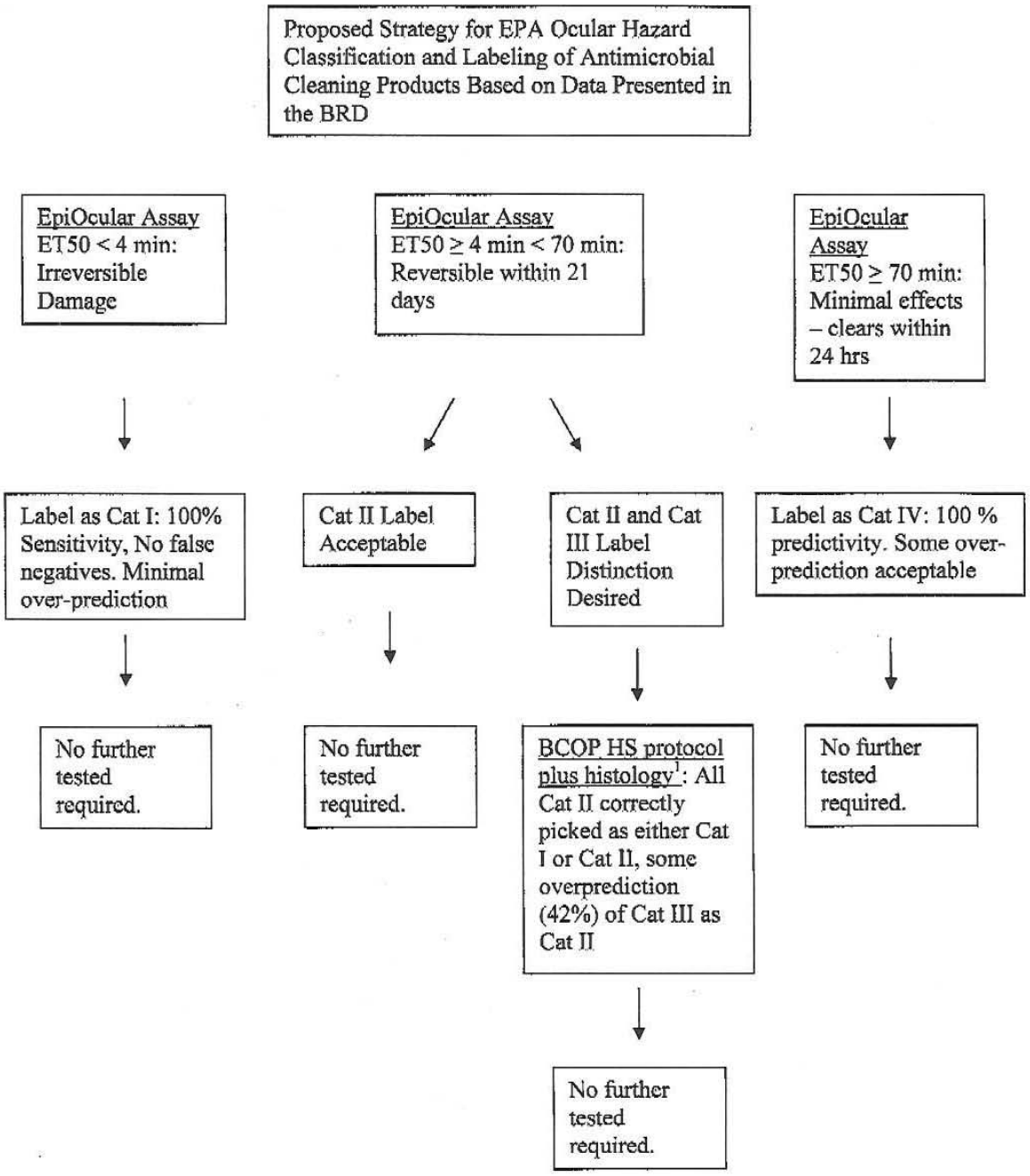


Figure 1. Proposed Strategy for EPA Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products Based on Data Presented in the BRD. [1]High solvent BCOP protocol with histology: BRD Table 6-50, P141.

13. May 2009

William Stokes, D.V.M., D.A.C.L.A.M.
Director NICEATM,
National Toxicology Program,
P.O. Box 12233, MD K2-16
Research Triangle Park, NC 27709

Dear Dr. Stokes,

This public comment is delivered in response to the Federal Register Notice Volume 74, Number 60, pages 14556 – 14557. It addresses the draft ICCVAM BRD on the Hen's Egg Test on the Chorio-Allantois Membrane (HET-CAM) (March/April 2009) with the current Status of In Vitro Test Methods for Identifying Low End Irritancy.

(http://iccvam.niehs.nih.gov/methods/ocutox/PeerPanel09.htm)

Introduction: In the Preface ICCVAM experts remarked on the lines 478 ff that the hen's egg test on the chorioallantoic membrane (HET-CAM) in a previous evaluation did not perform sufficiently to identify severe (irreversible) ocular irritants/corrosives using the EPA, United Nation Globally Harmonized System of Classification and Labeling Chemicals (GHS), and the European Union regulatory hazard classification system. This is in line with the findings of the German validation study (Spielmann et al. 1996, **24**, 741-858, Kalweit et al. 1990) and was the reason why the German outcome of the validation exercise proposed to use a combination of two methodologies to identify severe hazards more reliably. But proving such approach was not in the focus of the ICCVAM program.

ICCVAM now is reviewing the validation status of the HET-CAM for the identification of non-severe ocular irritants (that is, those that induce reversible ocular damage) and non-irritants. The Ocular Toxicity Working Group (OTWG) of ICCVAM and NICEATM has prepared a draft background review document (BRD) that summarizes the current status of this test methodology based on published and other submitted information.

General remarks: In its *Executive Summary* the OTWG experts have summarized that the CAM has been proposed as a model for a living membrane, since it comprises a functional vasculature, which does mean that the structural tissue damage induced by irritant chemicals can best be observed by the beginning of **vascular leakages (bleeding)** (line 575ff). A second additional information of structural damage induced by irritant chemicals can be the **coagulation** of structural and functional tissue components like proteins und carbohydrates (e.g. after protein denaturation, i.e. loss of functionality and solubility (which must not be irreversible per se). "**Coagulation**" is not equal to "**protein denaturation**". It can be the result of structural impairment (denaturation) of physiologically relevant gels accompanied by the loss of solubilisation and subsequent precipitation of structural constituents. This process can lead to cloudiness and/or opacity of originally clear and transparent gels playing obviously an important role in the visual process in the cornea.

Both processes tissue and cellular damage (bleeding) and coagulation ((cloudiness/opacity) play a role in the ocular tissues, the conjunctivae as well as in the corneal tissue. Coagulation as characteristic part of the corneal opacity can easily be observed and play a major role in estimating the impact, i.e. the severity and duration of especially strong irritants in the Draize scoring system. Coagulation does not reflect all types of corneal damage per se, and vice versa the damage of cellular matrices in the cornea ("area of depth and injury")

must not be accompanied by coagulation and consequently lead to opacity, these are two different qualities of damaging effects in the tissue. As a result there are two endpoints: i) vascular lyses, hemorrhages and bleeding that becomes visible, and ii) physicochemical damage and perturbation of transparent physiological gel matrices that become cloudy and opaque.

The confusion of terminologies that appears to still exist not only in the executive summary of this BRD and therefore might have influenced the outcome of this analysis is also characteristic for some older HET-CAM protocols, and in particular for the oldest version proposed by Lüpke et al.. There exist a number of protocols and modifications thereof that partly uses additional endpoints like **hyperemia** and/or **vascular lyses** that cannot be clearly identified or differentiated without using special microscopic equipment. But often enough this was not verified in the protocols. In our experience vascular lyses was not considered to be a valid separate endpoint but the prerequisite of the easily observed bleeding. At a later state vascular structures can disappear (in particular if certain types of surfactants have been applied). Similar observations showed that hyperemia cannot be differentiate without stereo microscope from slight diffuse bleeding. But hyperemia when it really occurs (mostly after treatment with slightly to non-irritant chemicals with particular properties) can be depending on the dose and time reversible phenomena of the capillary vasculature of the chorioallantois tissue.

Therefore it is not surprising that out of the large number of cited papers and procedures only few data sets seem to allow a comparison and subsequent biometrical analysis. As a result of this consideration there seem to be need to put together hemorrhages and vascular lyses for biometrical analysis and better leave out hyperemia for data analysis.

Validation Data Base (Line 587ff): The definition of in particular chemical classes more than product classes is a complex task. Accordingly the table in **Appendix A** is not very consistent. Since the biometrical analysis has been performed according to chemical or product classes it is may have an impact on the results. Some out of many examples might be given for illustration:

- **Anisole** is put into the classes; Ether and phenol, but
- **Phenol** itself is classified as alcohol, therefore it is not clear whether phenols are considered as alcohol.
- **Glycerin** (CASRN 56-81-5) is taken separately although it is a (German) synonym of
- **Glycerol** having the same CASRN. (*Compare also n-Butanol and Butanol*)
- **Potato Starch** is put into the class of “Hydrocarbons” although it belongs to the non-irritant Carbohydrates.
- **Potassium Laurate** seem to be the potassium **salt** of a fatty acid or carboxylic acid, but not a cationic surfactant, there are a lot of
- Inorganic and organic salts among the chemicals that are summarized as carboxylic acids although they might act as an anion, which is an essential difference.

Just to mention some aspects of classifying chemicals. The inorganic acids are not mentioned as such. This is of interest because strong acids organic and inorganic as well as alkalines must not be tested in vivo! - according to the OECD TG 405. A number of salts are classified as surfactants, may be because they act as such, but chemically they are organic salts like: Benzalkonium chloride and Sodium Dodecyl Sulfate.

This issue may hold true also for the other BRDs not reviewed in this paper. This list needs to be reviewed very critical for refining the results.

It seems to be more important from the viewpoint of applicability to sort the materials according to solubility in watery systems or in oil phases, as already done for the large document published in 1996 by Spielmann et al. in ATLA and several preceding papers, e.g. Kalweit et al. 1990, which contain all relevant parameters of the SOP which are missing in

the Appendix B1 and which might comprise the largest set of consistent data in this background review document.

This leads to the last remarks for the use of animals (BRD line 1900ff): In Appendix B1 it remains unclear how the days of embryonic development are counted. The process used to start after collecting the eggs, mostly with the artificial fertilization, and shipment to the laboratory, where then the start of the breeding is defined in a narrow slot before starting the breeding. Relevant are then the nine 24h-periods of breeding and development prior to testing in order to avoid the progress in the development of sensory nerve fibers.

The remarks collected and presented here comprise a brief summery and due to the time constrains for public comments not all possible and necessary comments.

Author: Wolfgang J.W. PAPE, *Raw Material Science, R&D Brands,* Beiersdorf AG, Unnastrasse 48, D-20253 Hamburg (Mail Box 562)

TNO Quality of Life

Nederlandse Organisatie voor toegepast-natuurwetenschappelijk onderzoek / Netherlands Organisation for Applied Scientific Research

Utrechtseweg 48
P.O. Box 360
3700 AJ Zeist
The Netherlands

www.tno.nl

T +31 30 694 41 44
F +31 30 695 72 24
info-voeding@tno.nl

Return address: P.O. box 360, 3700 AJ Zeist, The Netherlands

William Stokes, D.V.M, D.A.C.L.A.M.
Director, NICEATM/NIEHS
P.O. Box 12233, K2-16
Research Triangle, NC 27709

Date
May 14, 2009

Our reference
TAP 2009-091/prm-hok

E-mail
ruud.woutersen@tno.nl

Direct dialling
+31 30 694 45 03

Direct fax
+31 30 694 49 86

Attachments
- Individual BRD data with TNO comments and modified BRD data
- Individual *in vivo* and ICE data compound TNO-28
- Letter TAP 2005 plus annexes

Subject
ICE Report ICCVAM

Dear Dr. Stokes,

In reaction to the request for comments made public via the Federal Register notice, Vol. 74, No. 60, pages 14556-14557, March 31, 2009, the Dutch Research Organization TNO would like to forward the following comments and remarks concerning the ICE test method as described in the draft Proposed ICCVAM Test Method Recommendations: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches and its Draft Background Review Document.

One specific comment on the draft BRD of the ICE is:
Page 4-1, lines 897-905. The text stating the data sets and presence of individual *in vivo* data are incorrect and incomplete. The Prinsen (2005) data set containing 49 compounds is not mentioned. Furthermore, both the Prinsen (1996) and Prinsen (2005) data set contained in total 94 compounds/ formulations which were all tested in parallel with the *in vivo* eye irritation test in rabbits (OECD no. 405) or the *in vivo* skin irritation/ corrosion test (OECD no. 404; in case of skin corrosive compounds/formulations). **All the individual rabbit data were made available to ICCVAM**. This section should be corrected and if it has implications on the classifications already assigned they should be corrected accordingly.

From the document it appeared that ICCVAM mainly focused on the performance of the ICE with respect to three main classification systems (EPA, GHS and EU). The basis for these classifications is the individual *in vitro* and *in vivo* data of the compounds available and ICCVAM's interpretation with respect to classification. It is rather alarming and disappointing to notice that the database contains so many gaps in the *in vivo* classifications, whereas sufficient *in vivo* data are available for such a purpose. Furthermore, we noticed that mistakes have been made in some of the classifications. To give an example, compound TNO-28 has been classified as R41, whereas the individual *in vivo* scores (see attachment) are even below the threshold for classification as an irritant according to the EU- and the GHS-criteria. ICCVAM's argument for the missing classifications is that the data do not comply with the four criteria set by ICCVAM. These cases mainly concern studies which were terminated earlier than 21 days after treatment or compounds lacking an *in vivo* eye irritation study because of proven *in vivo* skin corrosivity. We

Nederlandse Organisatie voor toegepast-natuurwetenschappelijk onderzoek / Netherlands Organisation for Applied Scientific Research

Date
May 14, 2009

Our reference
TAP 2009-091/prm-hok

Page
2/4

consider these arguments refutable, especially because of the significance of *in vitro* methods in general for replacing the *in vivo* rabbit eye test. Furthermore, by ICCVAM's description of their own selection criteria the possibility exists not to comply with them and still have the data used if a good reason can be provided. It is, therefore, a missed opportunity if one does not investigate into more detail the data that are available and we urge ICCVAM to perform the evaluation the data gaps after being filled in by expert judgment of the available data. Using TNO's long experience in eye irritation (*in vivo* and *in vitro* since 1983), the individual data of these cases were reviewed and commented by us (see attachment ICE BRD March 2006, Appendix D1, pages D-5 to D-9 with TNO comments and attachment with data gaps completed by TNO). We kindly request ICCVAM to take the data and comments into consideration for an additional evaluation.

Another important issue we would like to address is the fact that the scientific world has always considered the rabbit *in vivo* eye irritation test to produce highly variable results, making it almost impossible for any alternative method to be accepted. We would like to illustrate this with two examples that can be found in numerous articles presented by scientist in the field of validation. From the Proceedings of the first World Congress on Alternatives held in 1993, Chapter A. **On Recognizing and Overcoming Barriers to the Acceptance of Alternative Methods** by Michael Balls and Julia Fentem, we would like to quote: "*In adequate analysis of data. It is very rare for any allowance to be made for the variability of the animal data, which are thus given a status which they do not deserve. They wrongly become the "true" values which the non-animal tests must struggle to reproduce. Also, insufficient allowance is made for the doubt which must be placed on values which fall within the barrier zones on both sides of category cut-off points. This is particular worrying when Cooper two-by-two way plots are used as a basis for establishing the sensitivity, specificity, predictivity and concordance of in vitro test data*".
From the publication in Toxicology *in vitro*, Volume 10 on Validation of alternative methods for toxicity testing (1996), the following: "*Computer simulations carried out by Bruner et al. have shown that, even if the alternative methods were perfectly reproducible (if their coefficients of variation were 0), the variability in the Draize scores alone would restrict the Pearson's correlation coefficients to the range 0.65 - 0.80 when the Draize scores are between 0 and 40 (typical of cosmetics ingredients)*". We would like to add that Draize scores between 0-40 in general represent the non to (mild) irritants.
The overall performance percentages obtained by the ICE (i.e. from all data sets combined) for non irritants were 66% (GHS), 78% (EPA) and 89% (EU). Considering the in house TNO ICE data set of 1996 and 2005 which were tested in parallel with the *in vivo* rabbit eye test and thus excluding a considerable part of the *in vivo* variability, even a higher performance of 92-94% (EU) was obtained for non irritants.

The reason why ICCVAM does not assess the ICE at its true value originates from its observation that the ICE at least in one case classified a compound as non irritant whereas *in vivo* it was a corrosive/severe irritant. This case and other cases all pertained solids and in one case a very sticky antifouling paint (TNO-94). The issue of solids or sticky substances is of particular importance, because it is one of the main reasons for the long and unsuccessful history of validation of alternatives for eye irritation. This has all to do with the fact that the standard *in vivo* rabbit eye irritation/corrosion test (OECD guideline no. 405) has no standardized exposure regimen. We do not know how much of the compound

Nederlandse Organisatie voor toegepast-natuurwetenschappelijk onderzoek / Netherlands Organisation for Applied Scientific Research

(solid, paste or liquid) and for how long the compound stays in contact with the eye; it can vary from minutes to hours. This kind of exposure is against all basic principles of toxicology testing, which is completely ignored in the evaluation by ICCVAM.
Further validation of alternatives would benefit tremendously if the **OECD guideline no. 405 would be modified to include a well-defined exposure regimen to the compound**. Moreover, the present exposure to solids can be considered highly unethical and cruel to the animals. Again we would like to quote the following from Michael Balls and Julia Fentem: "*To help bring this about (sic: more-rational attitudes to animal testing practices), the animal welfare movement must insist on the more-forceful application of animal protection laws such as Directive 86/609/EEC, which do not exclude regulatory animal testing from their Russell and Burch-inspired, three R's requirement that the use of animals be* ***reduced****, so that only the essential minimum number of animals are required, that the procedures necessarily applied to them be* ***refined****, so that they are more humane, and that they be* ***replaced*** *by non-animal methods wherever possible*". Surely, ICCVAM together with ECVAM and other organizations dealing with alternatives and animal welfare, such as the NCA (Dutch Centre for Alternatives) could combine forces to initiate such a process. If the other initiative of ICCVAM on the "Use of Topical Anesthetics, Systemic Analgesics, and 46 Humane Endpoints in Ocular Toxicity Testing to Avoid or Minimize Pain and Distress" would lead to any modification of OECD no. 405, an excellent opportunity would arise to also address the exposure issue which would even contribute more to the minimizing of pain and distress in the animals.

Having the irrefutable fact that higher performance values will never be realized with the present rabbit eye irritation test as the "Golden Standard", an organotypic model such as **the ICE should be allowed in a tiered testing approach for the screening of non-irritants**. We feel that this statement is supported by our data and perspective given in this public comment. In the event that reanalysis does not acceptably resolve the situation of the false negatives (true or not by the Golden Standard) the following changes in the prediction model and/or applicability domain could be considered: 1) the ICE criteria for classification as non irritant could be set at the lowest combination of effects possible, i.e. no effect on the three parameters measured, and 2) solids could be excluded or tested additionally with a longer exposure period, although we strongly oppose to idea that the ICE should try to match the unrealistic exposure regimen of the Draize Eye test. For other purposes, TNO has obtained (confidential) data with longer exposure periods from which it appears that a 30 second application to solids provides useful additional information.

We would like to finish this letter with a message to ICCVAM by using a final quote from the article **On Recognizing and Overcoming Barriers to the Acceptance of Alternative Methods** by M. Balls and Julia Fentem:
"All conceivable and practicable steps should be taken to make the formal acceptance and incorporation of non-animal toxicity test procedures into regulatory practice as smooth and rapid a process as is possible. The acceptance and incorporation process must not be rigid".

Nederlandse Organisatie voor toegepast-natuurwetenschappelijk onderzoek / Netherlands Organisation for Applied Scientific Research

Date
May 14, 2009

Our reference
TAP 2009-091/prm-hok

Page
4/4

We appreciate to have the opportunity to make these comments and kindly ask you to present them to the Expert Panel and general public before the meeting of 19-21 May 2009.

Yours faithfully,

Prof. Dr Ruud A. Woutersen
Deputy Head of the Business Unit Toxicology and Applied Pharmacology, TNO Quality of Life
Professor Translational Toxicology, Wageningen University

M.K. Prinsen
Toxicologist
Project Leader *in vitro* and *in vivo* eye irritation
Toxicology and Applied Pharmacology

In Vivo and In Vitro Comparison
Sorted by Reference

Substance/Product Name	CASRN	Concentration Tested	*In Vitro* Classification (GHS)	*In Vivo* Classification (GHS)	*In Vitro* Classification (EPA)	*In Vivo* Classification (EPA)	*In Vitro* Classification (EU)	*In Vivo* Classification (EU)	Reference
Acetone	67-64-1	undiluted	2A	2A	II	II	R36	R36	Balls et al (1995)
Ammonium nitrate	6484-52-2	undiluted	2B	2B	III	III	NI	R36	Balls et al (1995)
L-Aspartic acid	70-47-3	neat	2A	SCNM	II	SCNM	R36	SCNM	Balls et al (1995)
Benzalkonium chloride (1%)	8001-54-5	1%	2A	1	II	I	R36	R41	Balls et al (1995)
Benzalkonium chloride (5%)	8001-54-5	5%	1	1	I	I	R41	R41	Balls et al (1995)
Benzalkonium chloride (10%)	8001-54-5	10%	1	1	I	I	R41	R41	Balls et al (1995)
n-Butyl acetate	123-86-4	undiluted	2A	NI	II	III	R36	NI	Balls et al (1995)
Gammabutyrolactone	96-48-0	undiluted	2A	2A	II	II	R36	R36	Balls et al (1995)
Captan 90 concentrate	133-06-2	neat	2B	1	III	I	NI	R41	Balls et al (1995)
4-Carboxybenzaldehyde	619-66-9	neat	NI	2A	IV	II	NI	R36	Balls et al (1995)
Cetylpyridinium bromide (0 1%)	140-72-7	0 1%	2B	NI	III	III	NI	NI	Balls et al (1995)
Cetylpyridinium bromide (6%)	140-72-7	6%	2A	1	II	SCNM	R36	R41	Balls et al (1995)
Cetylpyridinium bromide (10%)	140-72-7	10%	2A	1	II	I	R36	R41	Balls et al (1995)
Chlorhexidine	55-56-1	neat	1	1	I	SCNM	R41	SCNM	Balls et al (1995)
Cyclohexanol	108-93-0	undiluted	1	1	I	I	R41	R41	Balls et al (1995)
Dibenzoyl-L-tartaric acid	2743-38-6	neat	1	1	I	SCNM	R41	R41	Balls et al (1995)
Dibenzyl phosphate	1623-08-1	neat	2A/2B	2A	II/III	II	R36	R36	Balls et al (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	undiluted	2A	2A	II	II	R36	SCNM	Balls et al (1995)
2,2-Dimethylbutanoic acid	595-37-9	undiluted	1	SCNM	I	I	R41	SCNM	Balls et al (1995)
2,5-Dimethylohexanediol	110-03-2	neat	2B	1	III	I	R36	R41	Balls et al (1995)
Ethanol	64-17-5	undiluted	1	2A	I	III	R41	NI	Balls et al (1995)
Ethyl acetate	141-78-6	undiluted	2A	NI	II	III	R36	NI	Balls et al (1995)
2-Ethyl-1-hexanol	104-76-7	undiluted	2A	2A	II	II	R36	R36	Balls et al (1995)
Ethyl-2-methylacetoacetate	609-14-3	undiluted	2B	2B	III	III	NI	NI	Balls et al (1995)
Ethyl trimethyl acetate	3938-95-2	undiluted	2B	NI	III	III	NI	NI	Balls et al (1995)
Fomesafen	72128-02-0	neat	2B	NI	III	III	NI	NI	Balls et al (1995)
Glycerol	56-81-5	undiluted	2B	NI	III	IV	NI	NI	Balls et al (1995)
n-Hexanol	111-27-3	undiluted	1	2A	I	II	R41	R36	Balls et al (1995)
Imidazole	288-32-4	neat	1	1	I	I	R41	R41	Balls et al (1995)
Isobutanol	78-83-1	undiluted	1	2A	I	II	R41	R36	Balls et al (1995)
Isopropanol	67-63-0	undiluted	1	2A	I	III	R41	SCNM	Balls et al (1995)
Maneb	12427-38-2	neat	NI	SCNM	IV	III	NI	SCNM	Balls et al (1995)
Methyl acetate	79-20-9	undiluted	1	2A	I	II	R41	R36	Balls et al (1995)
Methyl cyanoacetate	105-34-0	undiluted	NI	2A	IV	II	NI	R36	Balls et al (1995)
Methylcyclopentane	96-37-7	undiluted	NI	NI	IV	III	NI	NI	Balls et al (1995)
Methyl ethyl ketone	78-93-3	undiluted	1	2A	I	III	R41	R36	Balls et al (1995)
Methyl isobutyl ketone	108-10-1	undiluted	2A	NI	II	III	R36	NI	Balls et al (1995)
1-Napthaleneacetic acid	86-87-3	neat	2B	1	III	I	R36	SCNM	Balls et al (1995)
1-Napthaleneacetic acid, sodium salt	61-31-4	neat	1	1	I	I	R41	R41	Balls et al (1995)
n-Octanol	111-87-5	undiluted	2A	2B	II	II	R36	R36	Balls et al (1995)
Parafluoraniline	371-40-4	undiluted	1	SCNM	I	SCNM	R41	SCNM	Balls et al (1995)
Polyethylene glycol 400	25322-68-3	undiluted	2B	NI	III	IV	R36	NI	Balls et al (1995)
Potassium cyanate	590-28-3	neat	2B	SCNM	III	SCNM	R36	SCNM	Balls et al (1995)
Promethazine HCl	58-33-3	neat	1	1	I	I	R41	R41	Balls et al (1995)

In Vivo and In Vitro Comparison
Sorted by Reference

Substance/Product Name	CASRN	Concentration Tested	*In Vitro* Classification (GHS)	*In Vivo* Classification (GHS)	*In Vitro* Classification (EPA)	*In Vivo* Classification (EPA)	*In Vitro* Classification (EU)	*In Vivo* Classification (EU)	Reference
Pyridine	110-86-1	undiluted	1	1	I	I	R41	R41	Balls et al (1995)
Quinacrine	69-05-6	neat	2B	1	III	I	NI	R41	Balls et al (1995)
Sodium hydroxide (1%)	1310-73-2	1%	2A	2B	II	III	R36	R36	Balls et al (1995)
Sodium hydroxide (10%)	1310-73-2	10%	1	1	I	I	R41	R41	Balls et al (1995)
Sodium lauryl sulfate (3%)	151-21-3	3%	2B	NI	III	III	NI	NI	Balls et al (1995)
Sodium lauryl sulfate (15%)	151-21-3	15%	2B	1	III	I	R36	R36	Balls et al (1995)
Sodium oxalate	62-76-0	neat	2B	1	III	I	NI	R41	Balls et al (1995)
Sodium perborate, $4H_2O$	10486-00-7	neat	2B	1	III	I	NI	R41	Balls et al (1995)
Tetraaminopyrimidine sulfate	5392-28-9	neat	2B	NI	III	III	NI	NI	Balls et al (1995)
Toluene	108-88-3	undiluted	2A	NI	II	III	R36	NI	Balls et al (1995)
Trichloroacetic acid (3%)	76-03-9	3%	2A	NI	II	III	R36	NI	Balls et al (1995)
Trichloroacetic acid (30%)	76-03-9	30%	1	1	I	I	R41	R41	Balls et al (1995)
Triton X-100 (5%)	9002-93-1	5%	2A	2A	II	III	R36	NI	Balls et al (1995)
Triton X-100 (10%)	9002-93-1	10%	2A/2B	1	II/III	II	R36	R41	Balls et al (1995)
Tween 20	9005-64-5	undiluted	2B	NI	III	III	NI	NI	Balls et al (1995)
TNO-01 (Formulation-1)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-02 (Formulation-2)	n p	undiluted	2A	2A	II	II	R36	R36	Prinsen (1996)
TNO-03 (Pesticide-1)	n p	undiluted	NI	NI	IV	III	NI	NI	Prinsen (1996)
TNO-04 (Detergent-1)	n p	undiluted	2B	2A	III	III	NI	NI	Prinsen (1996)
TNO-05 (Silicone powder-1)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-06 (Lubricant)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-07 (Ink-1)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-08 (Ink-2)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-09 (Paint)	n p	undiluted	NI	NI	IV	II	NI	NI	Prinsen (1996)
TNO-10 (Silicone powder-2)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-11 (Sodium p-styrene sulfonate)	2695-37-6	undiluted	2A	SCNM	II	SCNM	R36	SCNM	Prinsen (1996)
TNO-12 (Formulation-3)	n p	undiluted	2A	NI	II	SCNM	R36	R36	Prinsen (1996)
TNO-13 (Pesticide-2)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-14 (Polydisaccharide)	n p	14 5%	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-15 (Polydisaccharide)	n p	50%	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-16 (Liquid nylon product)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-17 (Solvent-1)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-18 (Solvent-2)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-19 (Solvent-3)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-20 (Solvent-4)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-21 (Solvent-5)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-22 (Solvent-6)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-23 (Solvent-7)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-24 (Solvent-8)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-25 (Solvent-9)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-26 (Ink-3)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-27 (Thermal paper coating-1)	n p	undiluted	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-28 (Toilet cleaner-1)	n p	undiluted	2B	1	III	I	NI	R41	Prinsen (1996)
TNO-29 (Toilet cleaner-2)	n p	undiluted	2B	2A	III	III	NI	R36	Prinsen (1996)

In Vivo and In Vitro Comparison
Sorted by Reference

March 2006

Substance/Product Name	CASRN	Concentration Tested	*In Vitro* Classification (GHS)	*In Vivo* Classification (GHS)	*In Vitro* Classification (EPA)	*In Vivo* Classification (EPA)	*In Vitro* Classification (EU)	*In Vivo* Classification (EU)	Reference
TNO-30 (Pesticide-3)	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (1996)
TNO-31 (Sulfur)	7704-34-9	undiluted	NI	NI	IV	III	NI	NI	Prinsen (1996)
TNO-32 (Ink-4)	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (1996)
TNO-33 (Thermal paper coating-2)	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (1996)
TNO-34 (Detergent-2)	n p	undiluted	1	SCNM	I	SCNM	R41	SCNM	Prinsen (1996)
TNO-35 (Propyl-lactate)	616-09-1	undiluted	1	1	I	I	R41	R41	Prinsen (1996)
TNO-36 (Ethylhexyl lactate)	6283-86-9	undiluted	2A	SCNM	II	II	R36	SCNM	Prinsen (1996)
TNO-37 (Pesticide-4)	n p	undiluted	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-38 (Solvent-10)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-39 (Detergent-3)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-40 (Glycolbromoacetate form)	n p	undiluted	1	-	I	-	R41	R41 (SC)	Prinsen (1996)
TNO-41 (Amidosulfonic acid)	5329-14-6	undiluted	1	-	I	-	R41	R41 (SC)	Prinsen (1996)
TNO-42 (Glycolbromoacetate)	3785-34-0	85%	1	-	I	-	R41	R41 (SC)	Prinsen (1996)
TNO-43 (Monobromoacetic acid)	79-08-3	undiluted	1	-	I	-	R41	R41 (SC)	Prinsen (1996)
TNO-44 (Didecyldimethylammoniumchloride (23% in propyl glycol))	7173-51-5	23%	1	-	I	-	R41	R41 (SC)	Prinsen (1996)
Cetylpyridinium bromide (6%)	—	undiluted	1	1	I	SCNM	R41	R41	Prinsen (2000)
cyclohexylamino-functional PMS	—	undiluted	2A	-	II	-	R36	R36	Prinsen (2000)
decamethylcyclopentasiloxane	—	undiluted	NI	-	NI	-	NI	NI	Prinsen (2000)
Triton X-500 (5%)	—	undiluted	2B	-	III	-	NI	R36	Prinsen (2000)
TNO-45	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-46	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-47	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-48	n p	undiluted	2A	-	II	-	R36	R41 (SC)	Prinsen (2005)
TNO-49	n p	undiluted	1	-	I	-	R41	R41 (SC)	Prinsen (2005)
TNO-50	n p	undiluted	1	-	I	-	R41	R41 (SC)	Prinsen (2005)
TNO-51	n p	undiluted	1	-	I	-	R41	R41 (SC)	Prinsen (2005)
TNO-52	n p	undiluted	2B	2A	III	III	NI	R36	Prinsen (2005)
TNO-53	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-54	n p	undiluted	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-55	n p	undiluted	2B	2A	III	III	R36	R36	Prinsen (2005)
TNO-56	n p	undiluted	2B	2B	III	III	R36	NI	Prinsen (2005)
TNO-57	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-58	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-59	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-60	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-61	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-62	n p	undiluted	2B	NI	III	III	R36	NI	Prinsen (2005)
TNO-63	n p	undiluted	NI	NI	IV	III	NI	NI	Prinsen (2005)
TNO-64	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-65	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)

In Vivo and In Vitro Comparison
Sorted by Reference

Substance/Product Name	CASRN	Concentration Tested	*In Vitro* Classification (GHS)	*In Vivo* Classification (GHS)	*In Vitro* Classification (EPA)	*In Vivo* Classification (EPA)	*In Vitro* Classification (EU)	*In Vivo* Classification (EU)	Reference
TNO-66	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-67	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-68	n p	undiluted	2A	2A	II	II	R36	R36	Prinsen (2005)
TNO-69	n p	50%	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-70	n p	undiluted	2A	2A	II	III	R36	R36	Prinsen (2005)
TNO-71	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-72	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-73	n p	undiluted	1	2A	I	II	R41	R36	Prinsen (2005)
TNO-74	n p	undiluted	NI	NI	IV	III	NI	NI	Prinsen (2005)
TNO-75	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-76	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-77	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-78	n p	undiluted	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-79	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-80	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-81	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-82	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-83	n p	undiluted	2B	2B	III	III	NI	R36	Prinsen (2005)
TNO-84	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-85	n p	undiluted	2B	1	III	I	R36	R41	Prinsen (2005)
TNO-86	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-87	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-88	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-89	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-90	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-91	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-92	n p	undiluted	2B	1	III	I	R36	R41	Prinsen (2005)
TNO-93	n p	undiluted	2A	1	II	I	R36	R41	Prinsen (2005)
TNO-94	n p	undiluted	NI	1	NI	I	NI	R41	Prinsen (2005)
1-Butanol	71-36-3	undiluted	1	2A	I	II	R41	R41	Prinsen and Koëter (1993)
2-Butoxyethyl acetate	112-07-2	undiluted	2B	-	III	-	NI	NI	Prinsen and Koëter (1993)
2-Methoxyethanol	109-86-4	undiluted	2A	-	II	-	R36	R36	Prinsen and Koëter (1993)
Acetaldehyde	75-07-0	undiluted	2A	-	II	-	R36	R36	Prinsen and Koëter (1993)
Acetic acid	64-19-7	10%	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Benzalkonium chloride (100%)	8001-54-5	undiluted	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Brij 35	9002-92-0	undiluted	NI	-	IV	-	NI	NI	Prinsen and Koëter (1993)
Chloroform	67-66-3	undiluted	2A	-	II	-	R36	R36	Prinsen and Koëter (1993)
Dibutyltin dichloride	683-18-1	undiluted	1	-	I	-	R41	R41	Prinsen and Koëter (1993)
Dimethyl sulfoxide	67-68-5	undiluted	NI	2B	IV	III	NI	NI	Prinsen and Koëter (1993)
Glycerol	56-81-5	undiluted	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Mercury (II) chloride	7487-94-7	undiluted	1	-	I	-	R41	R41	Prinsen and Koëter (1993)
n-Hexane	110-54-3	undiluted	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Silver (I) nitrate	7761-88-8	3%	2B	-	III	-	NI	NI	Prinsen and Koëter (1993)
Sodium dodecyl sulfate	151-21-3	undiluted	2B	-	III	-	R41	R41	Prinsen and Koëter (1993)

In Vivo and In Vitro Comparison
Sorted by Reference

Substance/Product Name	CASRN	Concentration Tested	*In Vitro* Classification (GHS)	*In Vivo* Classification (GHS)	*In Vitro* Classification (EPA)	*In Vivo* Classification (EPA)	*In Vitro* Classification (EU)	*In Vivo* Classification (EU)	Reference
Sodium fluorescein	518-47-8	20%	NI	-	IV	-	NI	NI	Prinsen and Koëter (1993)
Sodium hydroxide	1310-73-2	1%	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Toluene	108-88-3	undiluted	2B	2B	III	III	NI	NI	Prinsen and Koëter (1993)
Triacetin	102-76-1	undiluted	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Tributyltin chloride	1461-22-9	undiluted	1	-	I	-	R41	R41	Prinsen and Koëter (1993)
Triethanolamine	102-71-6	undiluted	2B	NI	III	III	NI	NI	Prinsen and Koëter (1993)

SCNM - Study criteria not met
SC - Classification assigned on the basis of skin corrosion assay

Substance/Product Name	CASRN	Concentration Tested	*In Vitro Classification (GHS)*	*In Vivo Classification (GHS)*	*In Vitro* Classification (EPA)	*In Vivo* Classification (EPA)	*In Vitro Classification (EU)*	*In Vivo Classification (EU)*	Reference
Acetone	67-64-1	undiluted	2A	2A	II	II	R36	R36	Balls et al (1995)
Ammonium nitrate	6484-52-2	undiluted	2B	2B	III	III	NI	R36	Balls et al (1995)
L-Aspartic acid	70-47-3	neat	2A	SCNM	II	SCNM	R36	SCNM	Balls et al (1995)
Benzalkonium chloride (1%)	8001-54-5	1%	2A	1	II	I	R36	R41	Balls et al (1995)
Benzalkonium chloride (5%)	8001-54-5	5%	1	1	I	I	R41	R41	Balls et al (1995)
Benzalkonium chloride (10%)	8001-54-5	10%	1	1	I	I	R41	R41	Balls et al (1995)
n-Butyl acetate	123-86-4	undiluted	2A	NI	II	III	R36	NI	Balls et al (1995)
Gammabutyrolactone	96-48-0	undiluted	2A	2A	II	II	R36	R36	Balls et al (1995)
Captan 90 concentrate	133-06-2	neat	2B	1	III	I	NI	R41	Balls et al (1995)
4-Carboxybenzaldehyde	619-66-9	neat	NI	2A	IV	II	NI	R36	Balls et al (1995)
Cetylpyridinium bromide (0 1%)	140-72-7	0 1%	2B	NI	III	III	NI	NI	Balls et al (1995)
Cetylpyridinium bromide (6%)	140-72-7	6%	2A	1	II	SCNM	R36	R41	Balls et al (1995)
Cetylpyridinium bromide (10%)	140-72-7	10%	2A	1	II	I	R36	R41	Balls et al (1995)
Chlorhexidine	55-56-1	neat	1	1	I	SCNM	R41	SCNM	Balls et al (1995)
Cyclohexanol	108-93-0	undiluted	1	1	I	I	R41	R41	Balls et al (1995)
Dibenzoyl-L-tartaric acid	2743-38-6	neat	1	1	I	SCNM	R41	R41	Balls et al (1995)
Dibenzyl phosphate	1623-08-1	neat	2A/2B	2A	II/III	II	R36	R36	Balls et al (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	undiluted	2A	2A	II	II	R36	SCNM	Balls et al (1995)
2,2-Dimethylbutanoic acid	595-37-9	undiluted	1	1	I	I	R41	R41	Balls et al (1995)
2,5-Dimethylohexanediol	110-03-2	neat	2B	1	III	I	R36	R41	Balls et al (1995)
Ethanol	64-17-5	undiluted	1	2A	I	III	R41	NI	Balls et al (1995)
Ethyl acetate	141-78-6	undiluted	2A	NI	II	III	R36	NI	Balls et al (1995)
2-Ethyl-1-hexanol	104-76-7	undiluted	2A	2A	II	II	R36	R36	Balls et al (1995)
Ethyl-2-methylacetoacetate	609-14-3	undiluted	2B	2B	III	III	NI	NI	Balls et al (1995)
Ethyl trimethyl acetate	3938-95-2	undiluted	2B	NI	III	III	NI	NI	Balls et al (1995)
Fomesafen	72128-02-0	neat	2B	NI	III	III	NI	NI	Balls et al (1995)
Glycerol	56-81-5	undiluted	2B	NI	III	IV	NI	NI	Balls et al (1995)
n-Hexanol	111-27-3	undiluted	1	2A	I	II	R41	R36	Balls et al (1995)
Imidazole	288-32-4	neat	1	1	I	I	R41	R41	Balls et al (1995)
Isobutanol	78-83-1	undiluted	1	2A	I	II	R41	R36	Balls et al (1995)
Isopropanol	67-63-0	undiluted	1	2A	I	III	R41	SCNM	Balls et al (1995)
Maneb	12427-38-2	neat	NI	SCNM	IV	III	NI	SCNM	Balls et al (1995)
Methyl acetate	79-20-9	undiluted	1	2A	I	II	R41	R36	Balls et al (1995)
Methyl cyanoacetate	105-34-0	undiluted	NI	2A	IV	II	NI	R36	Balls et al (1995)
Methylcyclopentane	96-37-7	undiluted	NI	NI	IV	III	NI	NI	Balls et al (1995)
Methyl ethyl ketone	78-93-3	undiluted	1	2A	I	III	R41	R36	Balls et al (1995)
Methyl isobutyl ketone	108-10-1	undiluted	2A	NI	II	III	R36	NI	Balls et al (1995)
1-Napthaleneacetic acid	86-87-3	neat	2B	1	III	I	R36	SCNM	Balls et al (1995)
1-Napthaleneacetic acid, sodium salt	61-31-4	neat	1	1	I	I	R41	R41	Balls et al (1995)
n-Octanol	111-87-5	undiluted	2A	2B	II	II	R36	R36	Balls et al (1995)
Parafluoraniline	371-40-4	undiluted	1	1	I	SCNM	R41	R41	Balls et al (1995)
Polyethylene glycol 400	25322-68-3	undiluted	2B	NI	III	IV	R36	NI	Balls et al (1995)
Potassium cyanate	590-28-3	neat	2B	SCNM	III	SCNM	R36	SCNM	Balls et al (1995)
Promethazine HCl	58-33-3	neat	1	1	I	I	R41	R41	Balls et al (1995)

Substance/Product Name	CASRN	Concentration Tested	In Vitro Classification (GHS)	In Vivo Classification (GHS)	In Vitro Classification (EPA)	In Vivo Classification (EPA)	In Vitro Classification (EU)	In Vivo Classification (EU)	Reference
Pyridine	110-86-1	undiluted	1	1	I	I	R41	R41	Balls et al (1995)
Quinacrine	69-05-6	neat	2B	1	III	I	NI	R41	Balls et al (1995)
Sodium hydroxide (1%)	1310-73-2	1%	2A	2B	II	III	R36	R36	Balls et al (1995)
Sodium hydroxide (10%)	1310-73-2	10%	1	1	I	I	R41	R41	Balls et al (1995)
Sodium lauryl sulfate (3%)	151-21-3	3%	2B	NI	III	III	NI	NI	Balls et al (1995)
Sodium lauryl sulfate (15%)	151-21-3	15%	2B	2A	III	I	R36	R36	Balls et al (1995)
Sodium oxalate	62-76-0	neat	2B	1	III	I	NI	R41	Balls et al (1995)
Sodium perborate, 4H2O	10486-00-7	neat	2B	1	III	I	NI	R41	Balls et al (1995)
Tetraaminopyrimidine sulfate	5392-28-9	neat	2B	NI	III	III	NI	NI	Balls et al (1995)
Toluene	108-88-3	undiluted	2A	NI	II	III	R36	NI	Balls et al (1995)
Trichloroacetic acid (3%)	76-03-9	3%	2A	NI	II	III	R36	NI	Balls et al (1995)
Trichloroacetic acid (30%)	76-03-9	30%	1	1	I	I	R41	R41	Balls et al (1995)
Triton X-100 (5%)	9002-93-1	5%	2A	2A	II	III	R36	NI	Balls et al (1995)
Triton X-100 (10%)	9002-93-1	10%	2A/2B	1	II/III	II	R36	R41	Balls et al (1995)
Tween 20	9005-64-5	undiluted	2B	NI	III	III	NI	NI	Balls et al (1995)

Substance/Product Name	CASRN	Concentration Tested	*In Vitro Classification (GHS)*	*In Vivo Classification (GHS)*	*In Vitro* Classification (EPA)	*In Vivo* Classification (EPA)	*In Vitro Classification (EU)*	*In Vivo Classification (EU)*	Reference
TNO-01 (Formulation-1)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-02 (Formulation-2)	n p	undiluted	2A	2A	II	II	R36	R36	Prinsen (1996)
TNO-03 (Pesticide-1)	n p	undiluted	NI	NI	IV	III	NI	NI	Prinsen (1996)
TNO-04 (Detergent-1)	n p	undiluted	2B	2A	III	III	NI	NI	Prinsen (1996)
TNO-05 (Silicone powder-1)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-06 (Lubricant)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-07 (Ink-1)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-08 (Ink-2)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-09 (Paint)	n p	undiluted	NI	NI	IV	II	NI	NI	Prinsen (1996)
TNO-10 (Silicone powder-2)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-11 (Sodium p-styrene sulfonate)	2695-37-6	undiluted	2A	2A	II	SCNM	R36	R36	Prinsen (1996)
TNO-12 (Formulation-3)	n p	undiluted	2A /1	1	II	SCNM	R36/R41	R41	Prinsen (1996)
TNO-13 (Pesticide-2)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-14 (Polydisaccharide)	n p	14 5%	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-15 (Polydisaccharide)	n p	50%	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-16 (Liquid nylon product)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-17 (Solvent-1)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-18 (Solvent-2)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-19 (Solvent-3)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-20 (Solvent-4)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-21 (Solvent-5)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-22 (Solvent-6)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-23 (Solvent-7)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-24 (Solvent-8)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-25 (Solvent-9)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-26 (Ink-3)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-27 (Thermal paper coating-1)	n p	undiluted	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-28 (Toilet cleaner-1)	n p	undiluted	2B	2A	III	I	NI	NI/R36	Prinsen (1996)
TNO-29 (Toilet cleaner-2)	n p	undiluted	2B	2A	III	III	NI	NI	Prinsen (1996)

Substance/Product Name	CASRN	Concentration Tested	*In Vitro Classification (GHS)*	*In Vivo Classification (GHS)*	*In Vitro* Classification (EPA)	*In Vivo* Classification (EPA)	*In Vitro Classification (EU)*	*In Vivo Classification (EU)*	Reference
TNO-30 (Pesticide-3)	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (1996)
TNO-31 (Sulfur)	7704-34-9	undiluted	NI	NI	IV	III	NI	NI	Prinsen (1996)
TNO-32 (Ink-4)	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (1996)
TNO-33 (Thermal paper coating-2)	n p	undiluted	NI	NI	III	IV	NI	NI	Prinsen (1996)
TNO-34 (Detergent-2)	n p	undiluted	1	1	I	SCNM	R41	R41	Prinsen (1996)
TNO-35 (Propyl-lactate)	616-09-1	undiluted	1	1	I	I	R41	R41	Prinsen (1996)
TNO-36 (Ethylhexyl lactate)	6283-86-9	undiluted	2A	2A	II	II	R36	R36	Prinsen (1996)
TNO-37 (Pesticide-4)	n p	undiluted	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-38 (Solvent-10)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-39 (Detergent-3)	n p	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-40 (Glycolbromoacetate form)	n p	undiluted	1	1 (SC)	I	-	R41	R41 (SC)	Prinsen (1996)
TNO-41 (Amidosulfonic acid)	5329-14-6	undiluted	1	1 (SC)	I	-	R41	R41 (SC)	Prinsen (1996)
TNO-42 (Glycolbromoacetate)	3785-34-0	85%	1	1 (SC)	I	-	R41	R41 (SC)	Prinsen (1996)
TNO-43 (Monobromoacetic acid)	79-08-3	undiluted	1	1 (SC)	I	-	R41	R41 (SC)	Prinsen (1996)
TNO-44 (Didecyldimethylammoniumchloride (23% in propyl glycol))	7173-51-5	23%	1	-	I	-	R41	R41 (SC)	Prinsen (1996)
Cetylpyridinium bromide (6%)		undiluted	1	1	I	SCNM	R41	R41	Prinsen (2000)
cyclohexylamino-functional PMS		undiluted	2A	-	II	-	R36	R36	Prinsen (2000)
decamethylcyclopentasiloxane		undiluted	NI	-	NI	-	NI	NI	Prinsen (2000)
Triton X-500 (5%)		undiluted	2B	-	III	-	NI	R36	Prinsen (2000)
TNO-45	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-46	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-47	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-48	n p	undiluted	2A/1	1 (SC)	II	-	R36 /R41	R41 (SC)	Prinsen (2005)
TNO-49	n p	undiluted	1	1 (SC)	I	-	R41	R41 (SC)	Prinsen (2005)
TNO-50	n p	undiluted	1	1 (SC)	I	-	R41	R41 (SC)	Prinsen (2005)
TNO-51	n p	undiluted	1	1 (SC)	I	-	R41	R41 (SC)	Prinsen (2005)
TNO-52	n p	undiluted	2B	2A	III	III	NI /R36	R36	Prinsen (2005)
TNO-53	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-54	n p	undiluted	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-55	n p	undiluted	2B	2A	III	III	R36	R36	Prinsen (2005)
TNO-56	n p	undiluted	2B	2B	III	III	R36	NI (R36)	Prinsen (2005)
TNO-57	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-58	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-59	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-60	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-61	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-62	n p	undiluted	2B	NI	III	III	R36	NI	Prinsen (2005)
TNO-63	n p	undiluted	NI	NI	IV	III	NI	NI	Prinsen (2005)
TNO-64	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-65	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)

Substance/Product Name	CASRN	Concentration Tested	*In Vitro Classification (GHS)*	*In Vivo Classification (GHS)*	*In Vitro* Classification (EPA)	*In Vivo* Classification (EPA)	*In Vitro Classification (EU)*	*In Vivo Classification (EU)*	Reference
TNO-66	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-67	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-68	n p	undiluted	2A	2A	II	II	R36	R36	Prinsen (2005)
TNO-69	n p	50%	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-70	n p	undiluted	2A	2A	II	III	R36	R36	Prinsen (2005)
TNO-71	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-72	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-73	n p	undiluted	1	2A(1)	I	II	R41	R36(R41)	Prinsen (2005)
TNO-74	n p	undiluted	NI	NI	IV	III	NI	NI	Prinsen (2005)
TNO-75	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-76	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-77	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-78	n p	undiluted	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-79	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-80	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-81	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-82	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-83	n p	undiluted	2B	2B	III	III	NI /R36	R36	Prinsen (2005)
TNO-84	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-85	n p	undiluted	2B	1	III	I	R36	R41	Prinsen (2005)
TNO-86	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-87	n p	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-88	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-89	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-90	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-91	n p	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-92	n p	undiluted	2B	1	III	I	R36	R41*	Prinsen (2005)
TNO-93	n p	undiluted	2A	1	II	I	R36	R41	Prinsen (2005)
TNO-94	n p	undiluted	NI	1	NI	I	NI	R41**	Prinsen (2005)
1-Butanol	71-36-3	undiluted	1	2A	I	II	R41	R41	Prinsen and Koëter (1993)
2-Butoxyethyl acetate	112-07-2	undiluted	2B	-	III	-	NI	NI	Prinsen and Koëter (1993)
2-Methoxyethanol	109-86-4	undiluted	2A	-	II	-	R36	R36	Prinsen and Koëter (1993)
Acetaldehyde	75-07-0	undiluted	2A	-	II	-	R36	R36	Prinsen and Koëter (1993)
Acetic acid	64-19-7	10%	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Benzalkonium chloride (100%)	8001-54-5	undiluted	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Brij 35	9002-92-0	undiluted	NI	-	IV	-	NI	NI	Prinsen and Koëter (1993)
Chloroform	67-66-3	undiluted	2A	-	II	-	R36	R36	Prinsen and Koëter (1993)
Dibutyltin dichloride	683-18-1	undiluted	1	-	I	-	R41	R41	Prinsen and Koëter (1993)
Dimethyl sulfoxide	67-68-5	undiluted	NI	2B	IV	III	NI	NI	Prinsen and Koëter (1993)
Glycerol	56-81-5	undiluted	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Mercury (II) chloride	7487-94-7	undiluted	1	-	I	-	R41	R41	Prinsen and Koëter (1993)
n-Hexane	110-54-3	undiluted	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Silver (I) nitrate	7761-88-8	3%	2B	-	III	-	NI	NI	Prinsen and Koëter (1993)
Sodium dodecyl sulfate	151-21-3	undiluted	2B	-	III	-	R41	R41	Prinsen and Koëter (1993)

Substance/Product Name	CASRN	Concentration Tested	*In Vitro Classification (GHS)*	*In Vivo Classification (GHS)*	*In Vitro* Classification (EPA)	*In Vivo* Classification (EPA)	*In Vitro Classification (EU)*	*In Vivo Classification (EU)*	Reference
Sodium fluorescein	518-47-8	20%	NI	-	IV	-	NI	NI	Prinsen and Koëter (1993)
Sodium hydroxide	1310-73-2	1%	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Toluene	108-88-3	undiluted	2B	2B	III	III	NI	NI	Prinsen and Koëter (1993)
Triacetin	102-76-1	undiluted	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Tributyltin chloride	1461-22-9	undiluted	1	-	I	-	R41	R41	Prinsen and Koëter (1993)
Triethanolamine	102-71-6	undiluted	2B	NI	III	III	NI	NI	Prinsen and Koëter (1993)

SCNM - Study criteria not met
SC - Classification assigned on the basis of skin corrosion assay

* powder with entrapment in cul-de-sac

** paint adhering to the cornea

TNO-report

V 93.592 December 1993 Page 10

Compound TNO-20

Table 1 - Individual scores awarded to the ocular lesions elicited by

rabbit number	corneal opacity	iris effects	conjunctivae redness	conjunctivae chemosis	ocular discharge
			after one hour		
492	0	1	1	2	2
532	0	1	1	2	2
537	0	1	1	2	2
			3	6	6
			after 24 hours		
492	0	0	1	1	0
532	0	0	2	2	1
537	0	0	1	2	1
			4	5	2
			after 48 hours		
492	0	0	1	1	0
532	0	0	2	1	0
537	0	0	3[1]	2	0
			6	4	
			after 72 hours		
492	0	0	0	0	0
532	0	0	1	1	0
537	0	0	3[1]	2	0
			4	3	
			after 7 days		
492	0	0	0	0	0
532	0	0	0	0	0
537	0	0	3[1]	2	1
			after 14 and 21 days		
537	0	0	2	1	1[2]
			after 28 days		
537	0	0	0	0	0

[1] = ischemic necrosis on the nictitating membrane
[2] = white ocular discharge

The scores are explained in the appendix on the next page

217

Table 1 - Individual values for corneal swelling, corneal opacity and fluorescein retention values of the control and test eyes obtained with .....

Eye no.	Corneal Swelling	Corneal Opacity	Fluorescein Retention
		after 30 minutes	
1[1]	6.8	0.0	2.0
2	6.7	0.0	1.0
3	9.5	0.0	2.0
4	10.0	0.0	1.0
5	6.3	0.0	1.0
control	3.0	0.0	0.0
		after 75 minutes	
1	6.8	1.0	
2	8.3	0.5	
3	6.3	0.5	
4	8.3	0.0	
5	7.9	0.0	
control	-3.0	0.0	
		after 120 minutes	
1	10.2	1.0	
2	10.0	0.5	
3	11.1	0.5	
4	11.7	0.0	
5	9.5	0.0	
control	-1.5	0.0	
		after 180 minutes	
1	11.9	1.0	
2	10.0	0.5	
3	9.5	0.5	
4	15.0	0.0	
5	9.5	0.0	
control	-3.0	0.0	
		after 240 minutes	
1	13.6	1.0	
2	10.0	1.0	
3	9.5	0.5	
4	13.3	0.5	
5	14.3	0.0	
control	-3.0	0.0	

[1] eye no. 1 also showed some slight loosening of epithelial cells of the cornea

Table 2 - Mean values for corneal swelling, corneal opacity and fluorescein retention values of the test eyes treated wit[illegible] and the irritancy categories based on the maximum [illegible] scores, and the final EC-classification

Time intervals	Corneal Swelling	Corneal Opacity	Fluorescein Retention
30	7.9 (0.8)	0.0 (0.0)	1.4 (0.2)
75	7.5 (0.4)	0.4 (0.2)	
120	10.5 (0.4)	0.4 (0.2)	
180	11.2 (1.1)	0.4 (0.2)	
240	12.1 (1.0)	0.8 (0.1)	

parameter	maximum score	category
Corneal swelling	12.1	II
Corneal opacity	0.8	II
Fluorescein retention	1.4	II

(EC-)classification: ***NOT IRRITANT***

BORDERLINE CASE BETWEEN NON-IRRITATING AND IRRITATING

between brackets = standard error of the mean

TNO Quality of Life

Nederlandse Organisatie voor
toegepast-natuurwetenschappelijk
onderzoek/Netherlands Organisation
for Applied Scientific Research

Toxicology and Applied
Pharmacology
Location Zeist
Utrechtseweg 48
P.O. Box 360
3700 AJ Zeist
The Netherlands

www.tno.nl

T +31 30 694 41 44
F +31 30 695 72 24
infofood@voeding.tno.nl

Return address: Postbus 360, 3700 AJ, Zeist, The Netherlands

NTP/NICEATM
NIEHS
Attn. Dr. Raymond Tice
P.O. Box 12233, MD EC-17
Research Triangle Park, NC 27709
USA – BY AIRMAIL

Subject
Expert Panel Report ICCVAM/NICEATM

Date
August 8, 2005

Our reference
TAP-2005

Contact
Dr. R.A. Woutersen

E-mail
woutersen@voeding.tno.nl

Direct dialling
+31 30 694 4503

Direct fax
+31 30 696 02 64

Dear Sir,

In reaction to the request for comments made public via the Federal Register notice, Volume 70, No. 142, Tuesday, July 26, 2005/Notices, 43149, the Dutch Research Organization TNO would like to forward the following comments and remarks concerning the ICE test method described in the Addendum to "*In Vitro* Ocular Toxicity Draft Background Review Documents".

The inclusion of the additional data, forwarded by TNO beginning of this year, is highly appreciated. Because TNO has a longstanding experience with the screening of severe eye irritants for contract research, the additional data was forwarded to substantiate this particular application of the ICE. The data contained the full set of chemicals and/or formulations that was tested *in vitro* and *in vivo* over a period of several years. As we have experienced over the last 20 years of contract in *vivo* eye and skin irritation testing, about 10% of the compounds consist of eye/skin corrosives, of which almost all were screened by the ICE as indeed severely eye irritating. Therefore, we found it rather peculiar that eight severe eye irritating compounds were excluded form the reanalysis on the basis that insufficient *in vivo* data was provided to classify the compound according to the GHS classification system. All these compounds were corrosive in the *in vivo* skin irritation test, which was performed after the outcome of the ICE. The individual *in vivo* skin irritation data of these compounds were provided to ICCVAM. In full agreement with the guidelines, TNO decided to waive the *in vivo* eye irritation test in rabbits in these cases.
As the main purpose of the ICE (and any other *in vitro* eye irritation method) in contract research is to prevent severe irritants to be tested in the Draize eye test, it is rather paradoxical to see that all these actual cases of correctly identified severe eye irritants are not taken into account for the evaluation of this method.

Attachments
Article TIV (pdf : 4 pages)
ECETOC data (3 pages)

Nederlandse Organisatie voor
toegepast-natuurwetenschappelijk
onderzoek/Netherlands Organisation
for Applied Scientific Research

In contrast to what is taken by ICCVAM as the reason for exclusion, the GHS criteria clearly mentions "corrosive to skin" as one of the criteria for class I "irreversible eye effects". For the EPA classification, I cannot imagine that a skin corrosive is not assumed to be a category I compound for eye irritation.
Furthermore, two compounds from the EC/HO validation study study, i.e. *p*-fluoroaniline and 2,2-dimethyl butanoic acid, were excluded from the reanalysis. Both compounds were identified by the management of the EC/HO validation study (Balls et al., 1995) as R41 severely eye irritating on the basis of the individual *in vivo* data (ECETOC, Technical document no. 48: 2, June 1998; data attached). These two compounds were also correctly identified by the ICE and most other *in vitro* methods participating.
The *in vivo* classification was based on sound scientific judgment and it is unclear on which basis ICCVAM refuted this expert judgment. The probable reason for ICCVAM to exclude the compounds, may be that a 21-day observation period was not completed, is inadequate and, if so, demonstrates insufficient knowledge of the eye irritation process in rabbits. Moreover, the guidelines (at the time of testing) specified that the observation period should be long enough to evaluate the reversibility or irreversibility of the lesions.
The six rabbits treated with 2,2-dimethyl butanoic acid still showed slight to severe corneal opacity and neovascularization of the cornea at 14 days after treatment. Clearly, these lesions are not reversible within a 21-day observation period.
The same applies to *p*-fluoroaniline, causing moderate to severe corneal opacity and iritis score 2 (highest score possible; no reaction to light, haemorrhage, gross destruction). The test was terminated on day 3, which is in agreement with the current guidelines which mention that animals may be humanely sacrificed if the severity of the effects is considered too high.

On the basis of the above, TNO strongly requests ICCVAM to revise the present analyses with respect to the screening of severe irritants by inclusion of these ten cases. We have also concern about the fact that the data of the various studies performed with the ICE in different time periods are pooled for analyses and that the outcome of the individual studies is not discussed individually. Success or failure of *in vitro* methods has much to do with the setting in which the method is used and the way the *in vivo* data was obtained. Therefore, we advise ICCVAM to also mention and comment the successful use and strategy of the ICE by TNO for screening severe irritants.

With respect to the proposed Candidate substances (appendix V-A1), TNO would like to express reservations about the usefulness/appropriateness of such a list, containing only summary data. By now, after all the validation studies already carried out, we know that quite a different approach for validation is needed, including a meticulous test substance selection. With respect to this issue, we would like to draw your attention to the discussion article attached to this letter and which is currently in press in Toxicology In Vitro. This article deals, among other things, with the problem of using historical *in vivo* eye data for validation of *in vitro* test systems. We hope this article will contribute to the discussion concerning the validation process, i.e. not starting a new validation process before dealing with the basic issues of the *in vivo* test.

Nederlandse Organisatie voor
toegepast-natuurwetenschappelijk
onderzoek/Netherlands Organisation
for Applied Scientific Research

Date
August 8, 2005

Our reference
TAP-2005

Page
3/3

Attachments
Article TIV (pdf : 4 pages)
ECETOC data (3 pages)

We are looking forward ICCVAM's official reaction to our comments. In the mean time, TNO is, as always, available for additional information and discussion, if needed. In that respect, we were rather disappointed that we were not asked to comment on the above mentioned issue and, moreover, we were not informed of the availability of the reanalysis on the ICCVAM website.

Yours faithfully,

Dr Ruud A. Woutersen
Head of the Business Unit Toxicology and Applied Pharmacology, TNO Quality of Life

p-Fluoroaniline

Animal No. 4			Observation period (days) 1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	1	2	1	1	2	-	-	-	-	-	-	-
	Area involved	B	2	4	4	4	4	-	-	-	-	-	-	-
	(AxB) x 5		10	40	20	20	40	-	-	-	-	-	-	-
Iris		C	0	1	0	0	1	-	-	-	-	-	-	-
	C x 5		0	5	0	0	5	-	-	-	-	-	-	-
Conjunctiva	Redness	D	1	2	3	3	3	-	-	-	-	-	-	-
	Chemosis	E	2	3	2	2	3	-	-	-	-	-	-	-
	Discharge	F	1	1	1	2	2	-	-	-	-	-	-	-
	(D+E+F) x 2		8	12	12	14	16	-	-	-	-	-	-	-
Total			***18***	***57***	***32***	***34***	***61***	-	-	-	-	-	-	-

Animal No. 5			Observation period (days) 1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	1	1	1	1	3	-	-	-	-	-	-	-
	Area involved	B	2	4	4	4	4	-	-	-	-	-	-	-
	(AxB) x 5		10	20	20	20	60	-	-	-	-	-	-	-
Iris		C	0	0	0	0	2	-	-	-	-	-	-	-
	C x 5		0	0	0	0	10	-	-	-	-	-	-	-
Conjunctiva	Redness	D	1	2	2	2	3	-	-	-	-	-	-	-
	Chemosis	E	2	3	2	2	2	-	-	-	-	-	-	-
	Discharge	F	1	1	2	2	2	-	-	-	-	-	-	-
	(D+E+F) x 2		8	12	12	12	14	-	-	-	-	-	-	-
Total			***18***	***32***	***32***	***32***	***84***	-	-	-	-	-	-	-

Animal No. 6			Observation period (days) 1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	1	1	1	1	2	-	-	-	-	-	-	-
	Area involved	B	2	4	4	4	4	-	-	-	-	-	-	-
	(AxB) x 5		10	20	20	20	40	-	-	-	-	-	-	-
Iris		C	0	0	0	0	2	-	-	-	-	-	-	-
	C x 5		0	0	0	0	10	-	-	-	-	-	-	-
Conjunctiva	Redness	D	2	2	2	3	3	-	-	-	-	-	-	-
	Chemosis	E	2	3	2	2	3	-	-	-	-	-	-	-
	Discharge	F	1	1	1	2	3	-	-	-	-	-	-	-
	(D+E+F) x 2		10	12	10	14	18	-	-	-	-	-	-	-
Total			***20***	***32***	***30***	***34***	***68***	-	-	-	-	-	-	-

MMAS (Modified Maximum Average Score) (52+66+88+61+84+68) / 6 = 69.8

NB Test terminated at 3 days

opacity 28/18 = 1.6

2,2-Dimethyl Butanoic Acid

Source	Aldrich	Concentration tested	100 %
Specification		Volume tested	0.1 ml
CAS No.	595-37-9	No. of rabbits	6
Purity	96 %		
Product No.	D15,260-9		

			Observation period (days)											
Animal No.	1		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	1	-	1	1	2	-	2	-	-	-	2[a]	-
	Area involved	B	4	-	4	4	4	-	4	-	-	-	4	-
	(AxB) x 5		20	-	20	20	40	-	40	-	-	-	40	-
Iris		C	0	-	0	1	1	-	1	-	-	-	1	-
	C x 5		0	-	0	5	5	-	5	-	-	-	5	-
Conjunctiva	Redness	D	2	-	2	2	2	-	2	-	-	-	0	-
	Chemosis	E	1	-	1	0	0	-	0	-	-	-	0	-
	Discharge	F	1	-	1	1	2	-	1	-	-	-	0	-
	(D+E+F) x 2		8	-	8	6	8	-	6	-	-	-	0	-
Total			***28***	-	***28***	***31***	***53***	-	***51***	-	-	-	***45***	-

			Observation period (days)											
Animal No.	2		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	2	-	2	1	1	-	2	-	-	-	1[a]	-
	Area involved	B	4	-	4	4	4	-	4	-	-	-	3	-
	(AxB) x 5		40	-	40	20	20	-	40	-	-	-	15	-
Iris		C	1	-	0	0	0	-	0	-	-	-	0	-
	C x 5		5	-	0	0	0	-	0	-	-	-	0	-
Conjunctiva	Redness	D	2	-	2	2	2	-	2	-	-	-	0	-
	Chemosis	E	1	-	1	0	0	-	0	-	-	-	0	-
	Discharge	F	1	-	0	1	1	-	0	-	-	-	0	-
	(D+E+F) x 2		8	-	6	6	6	-	4	-	-	-	0	-
Total			***53***	-	***46***	***26***	***26***	-	***44***	-	-	-	***15***	-

			Observation period (days)											
Animal No.	3		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	1	-	1	1	1	-	1	-	-	-	1	-
	Area involved	B	4	-	4	4	4	-	4	-	-	-	2	-
	(AxB) x 5		20	-	20	20	20	-	20	-	-	-	10	-
Iris		C	0	-	0	0	0	-	0	-	-	-	0	-
	C x 5		0	-	0	0	0	-	0	-	-	-	0	-
Conjunctiva	Redness	D	2	-	2	2	2	-	1	-	-	-	0	-
	Chemosis	E	3	-	1	0	0	-	0	-	-	-	0	-
	Discharge	F	1	-	0	1	1	-	0	-	-	-	0	-
	(D+E+F) x 2		12	-	6	6	6	-	2	-	-	-	0	-
Total			***32***	-	***26***	***26***	***26***	-	***22***	-	-	-	***10***	-

2,2-Dimethyl Butanoic Acid

			Observation period (days)											
Animal No.	4		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	2	-	2	2	2	-	2	-	-	-	1[a]	-
	Area involved	B	4	-	4	4	4	-	4	-	-	-	4	-
	(AxB) x 5		40	-	40	40	40	-	40	-	-	-	20	-
Iris		C	1	-	1	1	1	-	1	-	-	-	0	-
	C x 5		5	-	5	5	5	-	5	-	-	-	0	-
Conjunctiva	Redness	D	2	-	2	2	2	-	2	-	-	-	0	-
	Chemosis	E	2	-	1	1	1	-	0	-	-	-	0	-
	Discharge	F	1	-	1	2	2	-	1	-	-	-	0	-
	(D+E+F) x 2		10	-	8	10	10	-	6	-	-	-	0	-
Total			***55***	-	***53***	***55***	***55***	-	***51***	-	-	-	***20***	-

			Observation period (days)											
A. .ial No.	5		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	1	-	1	1	2	-	3[a]	-	-	-	3[a]	-
	Area involved	B	4	-	4	4	4	-	4	-	-	-	4	-
	(AxB) x 5		20	-	20	20	40	-	60	-	-	-	60	-
Iris		C	0	-	0	0	1	-	1	-	-	-	2	-
	C x 5		0	-	0	0	5	-	5	-	-	-	10	-
Conjunctiva	Redness	D	2	-	2	2	2	-	2	-	-	-	1	-
	Chemosis	E	2	-	1	0	1	-	1	-	-	-	0	-
	Discharge	F	1	-	1	0	2	-	1	-	-	-	0	-
	(D+E+F) x 2		10	-	8	4	10	-	8	-	-	-	2	-
Total			***30***	-	***28***	***24***	***55***	-	***73***	-	-	-	***72***	-

			Observation period (days)											
Animal No.	6		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	2	-	2	2	2	-	1	-	-	-	2[a]	-
	Area involved	B	4	-	4	4	4	-	4	-	-	-	4	-
	(AxB) x 5		40	-	40	40	40	-	20	-	-	-	40	-
Iris		C	1	-	1	1	1	-	0	-	-	-	0	-
	C x 5		5	-	5	5	5	-	0	-	-	-	0	-
Conjunctiva	Redness	D	2	-	2	2	2	-	1	-	-	-	0	-
	Chemosis	E	2	-	2	1	1	-	0	-	-	-	0	-
	Discharge	F	1	-	1	1	1	-	0	-	-	-	0	-
	(D+E+F) x 2		10	-	10	8	8	-	2	-	-	-	0	-
Total			***55***	-	***55***	***53***	***53***	-	***22***	-	-	-	40	-

MMAS (Modified Maximum Average Score) (53+26+26+55+55+53) / 6 = 44.7

[a] Corneal vascularisation
NB Test Terminated at 14 days

Sodium Lauryl Sulphate

Source	Sigma	Concentration tested	15.0 %
Specification		Volume tested	0.1 ml
CAS No.	151-21-3	No. of rabbits	6
Purity	98 %		
Product No.	L-4509		

			Observation period (days)											
Animal No.	1		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	-	-	2	2	1	-	0	-	0	-	-	-
	Area involved	B	-	-	4	3	2	-	0	-	0	-	-	-
	(AxB) x 5		-	-	40	30	10	-	0	-	0	-	-	-
Iris		C	-	-	0	0	0	-	1	-	0	-	-	-
	C x 5		-	-	0	0	0	-	5	-	0	-	-	-
Conjunctiva	Redness	D	-	-	2	2	2	-	2	-	0	-	-	-
	Chemosis	E	-	-	2	1	1	-	1	-	0	-	-	-
	Discharge	F	-	-	3	2	1	-	0	-	0	-	-	-
	(D+E+F) x 2		-	-	14	10	8	-	6	-	0	-	-	-
Total			-	-	***54***	***40***	***18***	-	***11***	-	***0***	-	-	-

			Observation period (days)											
Animal No.	2		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	-	-	2	2	1	-	0	-	-	-	-	-
	Area involved	B	-	-	3	2	1	-	0	-	-	-	-	-
	(AxB) x 5		-	-	30	20	5	-	0	-	-	-	-	-
Iris		C	-	-	1	1	0	-	0	-	-	-	-	-
	C x 5		-	-	5	5	0	-	0	-	-	-	-	-
Conjunctiva	Redness	D	-	-	2	2	1	-	0	-	-	-	-	-
	Chemosis	E	-	-	2	1	1	-	0	-	-	-	-	-
	Discharge	F	-	-	2	1	1	-	0	-	-	-	-	-
	(D+E+F) x 2		-	-	12	8	6	-	0	-	-	-	-	-
Total			-	-	***47***	***33***	***11***	-	***0***	-	-	-	-	-

			Observation period (days)											
Animal No.	3		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	-	-	2	2	1	-	0	-	0	-	-	-
	Area involved	B	-	-	4	2	1	-	0	-	0	-	-	-
	(AxB) x 5		-	-	40	20	5	-	0	-	0	-	-	-
Iris		C	-	-	2	2	1	-	0	-	0	-	-	-
	C x 5		-	-	10	10	5	-	0	-	0	-	-	-
Conjunctiva	Redness	D	-	-	2	2	2	-	0	-	0	-	-	-
	Chemosis	E	-	-	3	2	2	-	1	-	0	-	-	-
	Discharge	F	-	-	3	2	1	-	0	-	0	-	-	-
	(D+E+F) x 2		-	-	16	12	10	-	2	-	0	-	-	-
Total			-	-	***66***	***42***	***20***	-	***2***	-	***0***	-	-	-

Sodium Lauryl Sulphate

			Observation period (days)											
Animal No.	4		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	-	-	2	1	1	-	0	-	-	-	-	-
	Area involved	B	-	-	3	2	1	-	0	-	-	-	-	-
	(AxB) x 5		-	-	30	10	5	-	0	-	-	-	-	-
Iris		C	-	-	2	1	1	-	0	-	-	-	-	-
	C x 5		-	-	10	5	5	-	0	-	-	-	-	-
Conjunctiva	Redness	D	-	-	2	2	2	-	0	-	-	-	-	-
	Chemosis	E	-	-	2	1	1	-	0	-	-	-	-	-
	Discharge	F	-	-	2	1	1	-	0	-	-	-	-	-
	(D+E+F) x 2		-	-	12	8	8	-	0	-	-	-	-	-
Total			-	-	***52***	***23***	***18***	-	***0***	-	-	-	-	-

			Observation period (days)											
Animal No.	5		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	-	-	2	1	1	-	1	-	1	-	1	1
	Area involved	B	-	-	4	2	1	-	1	-	1	-	1	1
	(AxB) x 5		-	-	40	10	5	-	5	-	5	-	5	5
Iris		C	-	-	2	1	1	-	0	-	0	-	2	0
	C x 5		-	-	10	5	5	-	0	-	0	-	10	0
Conjunctiva	Redness	D	-	-	3	2	2	-	2	-	1	-	1	0
	Chemosis	E	-	-	3	2	2	-	2	-	1	-	1	0
	Discharge	F	-	-	3	2	2	-	2	-	1	-	1	0
	(D+E+F) x 2		-	-	18	12	12	-	12	-	6	-	6	0
Total			-	-	***68***	***27***	***22***	-	***17***	-	***11***	-	***21***	***5***

			Observation period (days)											
Animal No.	6		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	-	-	2	2	2	-	0	-	0	-	0	-
	Area involved	B	-	-	4	2	1	-	0	-	0	-	0	-
	(AxB) x 5		-	-	40	20	10	-	0	-	0	-	0	-
Iris		C	-	-	2	1	1	-	1	-	0	-	0	-
	C x 5		-	-	10	5	5	-	5	-	0	-	0	-
Conjunctiva	Redness	D	-	-	3	2	2	-	1	-	1	-	0	-
	Chemosis	E	-	-	3	2	2	-	0	-	0	-	0	-
	Discharge	F	-	-	3	2	1	-	0	-	0	-	0	-
	(D+E+F) x 2		-	-	18	12	10	-	2	-	2	-	0	-
Total			-	-	***68***	***37***	***25***	-	***7***	-	***2***	-	***0***	-

MMAS (Modified Maximum Average Score) (54+47+66+52+68+68) / 6 = 59.2

Subject: FR Notice Comments - 74FR14556 - Ocular Peer Panel Meeting
Date: Friday, May 15, 2009 2:00 PM

Below is the result of your feedback form. It was submitted by
() on Friday, May 15, 2009 at 14:00:03

Comment_date: May 15, 2009

Prefix: Dr.

FirstName: Robert

LastName: Rapaport

Degree: Ph.D.

onBehalfOf: yes

Title: Associate Director

Department: Product Safety and Regulatory Affairs

Company: The Procter & Gamble Company

Country: USA

Phone:

EMail:

Comments: May 14, 2009
William Stokes, D.V.M. D.A.C.L.A.M.
Director, NICEATM
National Toxicology Program
P.O. Box 12233, K2-16
Research Triangle Park, NC 27709

Dear Dr. Stokes,

This public comment is provided in response to Federal Register Notice Volume 74, Number 60, pages 14556-14557 requesting comments in the context of the public meeting of an independent scientific peer review panel on alternative ocular safety testing methods that will take place on May 19-21, 2009.

The Procter & Gamble Company fully supports the advancement of alternatives to animal testing. As such, it commends ICCVAM for undertaking this current activity on evaluation of in vitro eye irritation assays validation status and their use in tiered testing strategies for anti-microbial products. It is also noteworthy that the Top/Down-Bottom/Up approach: eye irritation testing strategy to reduce and replace in vivo studies was recently accepted for publication in the peer-reviewed scientific literature (Scott et al. Toxicology In Vitro, accepted for publication).

However, given the extensive industry experience and collective historical data on LVET that exist and which are not fully reflected in the LVET Summary Review Document (SRD), the Procter & Gamble Company would like to raise concerns on this and provide additional technical perspective for consideration by the peer review panel.

This public comment is specifically related to use of historical and published LVET data to support use of this assay as an acceptable in vivo reference standard against which to compare in vitro assays used in a tiered testing strategy for anti-microbial products. It will seek to provide additional information and perspective on specific comments made in the draft ICCVAM SRD: The Low Volume Eye Test (LVET) dated April 1, 2009 that was published on the NICEATM-ICCVAM website.

It is structured to provide a summary of its conclusions followed by specific detailed responses for your consideration to identified focus areas mentioned at different points throughout the LVET SRD on which questions are raised concerning use of LVET as an acceptable reference standard in the context of this ocular methods/approaches review. Each focus area addressed includes line references within the LVET SRD where the questions arise).

Summary of public comment

There exists an extensive historical LVET database that supports use of such existing LVET data as an appropriate in vivo reference standard against which to compare in

vitro assays within the context of the current ICCVAM review “Use of In Vitro Methods in a Tiered Approach for Ocular Hazard Identification of Anti-Microbial Products”. Furthermore, this dataset provides data for several characteristics of the assay that are key to scientific acceptance of historical and available LVET data. This is in the context of domains of applicability for which the data support its use in a WoE approach as a valid and relevant predictor of eye irritation and as such in vivo reference standard against which to compare in vitro assays. These are:

- Anatomical and physiological basis for choice of 10 uL as an appropriate dose volume
- Ability of 10 uL dose volume to effectively discriminate between materials of different eye irritancy potential
- Ability of LVET to detect the range of ocular responses from innocuous to severe
- Ability of LVET to correctly predict known severe human eye irritants
- Over-prediction of the human response by LVET, but to a lesser extent than the Draize test, thereby remaining a conservative evaluation of eye irritation potential
- Correlation of LVET dosing procedure with the human response in clinical studies
- Correlation of LVET data and human experience data from industrial accidents and consumer accidental exposure for the same consumer products

Most recently, use of LVET as an appropriate in vivo reference standard against which the Isolated Chicken Eye (ICE) Test was compared to establish the latter as a suitable in vitro assay to determine eye irritation potential of household cleaning products was accepted for publication in the peer reviewed scientific literature (Schutte et al. Regulatory Toxicology and Pharmacology, accepted for publication).

Comments on identified focus areas within the LVET SRD

1. The nature and range of irritancy of substances tested in LVET. It is reported in the SRD that the majority of LVET data has been generated on surfactant-based mixtures or products which produce only a mild irritant response or no response [lines 280-281].

Furthermore, it is reported that there is no information on the performance of known corrosives in the LVET [lines 285-286].

It is recognised that a significant amount of the historical LVET data available is for surfactant-based materials and surfactant-based products. This reflects the original purpose for development of the LVET as a modified Draize test that better predicts the human response from accidental ocular exposure to detergent and cleaning products. In the development of LVET and its use as the in vivo reference standard in the mechanisms of eye irritation work conducted by Maurer, Jester and others, the range of materials tested in LVET extends well beyond only surfactant-based materials and surfactant-based products.

The work conducted by Griffith et al. (1) in the early stages of its development used a range of chemicals including solvents, acids, alkalis, surfactants, aldehydes, amines and general chemicals that were grouped into four irritancy categories (innocuous/non-irritant, moderate, substantial and severe irritant/corrosive) based on human experience derived from literature e.g. Grants Toxicology of the eye (2), occupational incidents within the industrial setting, Poison Control Centre (PCC) data and reports of consumer exposures to detergent and cleaning products. What should not be in question is that known human ocular corrosives/severe irritants were included in this chemical dataset namely acetic acid (10%), NaOH (10%), Ca(OH)2 (100%) and formaldehyde (38%) which have all been identified as ocular corrosives/severe irritants in the human eye. For all of these chemicals, both the LVET and Draize in this study identified them as ocular corrosives/severe irritants.

Similarly, the chemical set used in the mechanisms of eye irritation work by Maurer et al. (3) and Jester (4) included acids, alkalis, alcohols, ketones, peroxides, aldehydes, bleaches, solvents, peroxides as well as surfactants (anionic, non-ionic, cationic). Several of these materials were identified by LVET as being severe eye irritants, some of which again are known human ocular corrosives (e.g. NaOH).

Furthermore, the publication by Cormier et al. (5)

identified 70 parallel LVET and Draize tests conducted on 53 surfactant-based detergent and cleaning and personal care products. Within this historical dataset, LVET identified products that were not classified, irritant and severe irritant. Given the nature of the products, it is logical and expected that most of these products were identified as NC. However, it is important to recognise that LVET was capable of identifying products within this dataset that did merit irritant classifications.

From this it can be concluded that the retrospective historical and available LVET dataset is: 1) based on a range of substances from different chemical classes and consumer products from different product categories; 2) spans the range of irritancy from innocuous to severe and 3) includes known human ocular corrosives.

2. Comparative traditional Draize rabbit data with which to evaluate the accuracy of the LVET are only available for limited types and numbers of substances (i.e. surfactant-containing personal and household cleaning products and comparative human data from clinical studies and accidental exposures proposed to support its accuracy are largely with substances that are mild or non-irritating [lines 300-305].

Parallel datasets that compare the traditional Draize test with LVET for the same substances are available for both surfactant-based and non-surfactant-based substances/products. Such datasets are reported in the publications by Griffith et al. (1) for a range of chemicals that include solvents, acids, alkalis, surfactants, aldehydes, amines and general chemicals and by Cormier et al. (5), Freeberg et al. (6, 7, 8) and Gettings et al. (9, 10) for surfactant-based products.

Indeed, it is the original work by Griffith et al. (1) that investigated dose response characteristics with increasing dose volumes (10 uL, 30 uL, 50 uL and 100 uL). These investigators demonstrated statistically that 10 uL was the most effective dose volume for discriminating between substances with different levels of irritancy (defined by National Academy of Sciences (NAS) and Federal Hazardous Substances Act (FHSA) criteria) from innocuous to severe. Furthermore this study identified that: 1) a 10

uL dose volume is capable of detecting the same range of tissues (cornea, iris, conjunctiva) and severity of effects as in the Draize test; 2) correctly classified materials identified as non-hazardous and hazardous (except SLS (40%)) in humans and 3) demonstrated that 30 uL and 100 uL dose volume in rabbits over-classified materials identified as non-hazardous in humans.

The Cormier et al. (5) work used a historical dataset of 70 Draize-LVET studies on surfactant-based products to evaluate, by regression analysis, the linear relationship of LVET to the Draize test. This work established that LVET gives responses that are linearly correlated to the Draize test. Within this historical dataset, LVET identified products that were not classified, irritant and severe irritants.
The studies by Freeberg et al. used parallel Draize-LVET datasets that were compared with clinical data for the same surfactant-based products in one study (7) and with human experience from industrial accidents and follow-up of consumer accidental exposures in two other studies (6, 8). In their correlation of the Draize-LVET dataset to clinical data (7), four different surfactant-based consumer products (undiluted liquid fabric softener, 20% liquid shampoo, 10% liquid hand soap and 4% liquid laundry detergent) were dosed at both 10 uL (LVET dosing) and 100 uL (Draize dosing) in the rabbit and humans. Though formal classifications were not calculated for the products at the time of this study, retrospective classification has identified that rabbit LVET and Draize tests both identified the liquid laundry detergent tested undiluted as a severe eye irritant (R41). This further addresses a comment in the SRD that only non- or only mild irritants have been tested in LVET.

In the Freeberg et al. work that used parallel Draize-LVET datasets compared with human experience from industrial accidents/follow-up of consumer accidental exposures, 29 detergent and cleaning products were included in one study (6) and 14 detergent and cleaning products and personal care products in a second study (8). The formulations that were included in these evaluations were reflective of product formulations in development and/or marketed to consumers and were identified as mild-moderate irritants on the basis of Maximum Average Scores (MAS) and Time-To-Clear for ocular responses.

From this it can be concluded that several historical parallel LVET-Draize datasets are available and published in the scientific literature that cover surfactant-based materials and products as well as different classes of chemicals including solvents, acids, alkalis, surfactants, aldehydes, amines and general chemicals. In all of these rabbit LVET-Draize parallel datasets, the Draize test produced more severe responses in terms of ocular tissues involved (cornea, iris, conjunctiva), severity and persistence of ocular effects than LVET. Since this response addresses availability of rabbit LVET-Draize datasets, correlation to the human response is not discussed here but is addressed in point 4 below. The data from these studies also support the conclusion that the range of irritancy of materials addressed in these historical parallel LVET-Draize datasets is from innocuous to severe.

3. A comparison of the substances that have been classified by the Draize rabbit eye test as ocular corrosives or severe irritants that have also been tested in the LVET indicates that the LVET routinely under-predicts the ocular corrosive or severe irritant response in the Draize in many cases by more than one hazard category. This is illustrated by the results of Gettings et al (1996) in their evaluation of 25 surfactant-containing formulations [lines 422-427].
The above statement makes the assumption that the Draize classification is the correct classification for the surfactant-containing formulations tested by Gettings et al. (9, 10) and does not take into consideration that the Draize classification for these surfactant-containing products could be over-predictions. Some examples of the surfactant-containing formulations classified as EPA Category 1 (corrosive) by the Draize test from the Gettings et al. study (9) are a baby shampoo, bath foam, gel cleanser and facial cleansing foam. Such products included in this study were not prototypes nor were they products rejected for marketing due to excessive eye irritation but were formulations that were representative of those product types in the marketplace at that time for which accidental eye exposure would have undoubtedly been expected to occur. Formulation details for these products are publicly available and a review of these formulations

based on their chemical composition would not indicate that these products would be corrosive to the eye. Furthermore, a corrosive classification is not borne out by the human experience that has occurred over many years for these types of surfactant-containing cosmetic products marketed by several companies. It is reasonable to expect that if a baby shampoo was truly corrosive then marketing of such a product over many years by several companies would have resulted in reports of serious eye effects from accidental eye exposure being detected in the human experience. This is simply not the case.

It is also interesting to note that it is often the result of a single in vivo assay that is used in the correlation of in vitro assay data with the in vivo reference standard. This does not take into account the inherent variability of the in vivo test since without the results of multiple tests it is difficult to assess test variability. One of the few studies to take this into account is the CTFA Phase III study which used bootstrap re-sampling to estimate the within group variability for each test material. Since the Draize test has evolved over time from a 6 animal test to the current 3 animal test it is possible, from the CTFA Phase III study, for each test material, to break down the in vivo 6 animal tests into 20 unique combinations of 3 animal groups. It is then possible to determine a classification for each 3 animal group and identify the number of sub-groups in each classification class. To illustrate this point, test material HZE (gel cleanser) has been chosen from the CTFA Phase III study.

This test material is identified in the LVET SRD in Table 4-3 [line 449] as having a classification of EPA Category I based on the Draize test and EPA Category III based on LVET. An analysis of the 20 unique combinations of 3 animal groups from the 6 animal Draize test for this material identifies that 10 of the possible 20 combinations yield a classification of EPA Category I (corrosive) but interestingly the other 10 possible combinations yield a classification of EPA Category IV (non-irritant). This demonstrates that if a single 3 animal Draize test had been conducted there would be an equal chance of identifying the gel cleanser as a non-irritant or corrosive. Conducting this same exercise for test material HZE (gel cleanser) tested in the 6 animal LVET identifies all 20 unique combinations of 3 animal groups as having a classification of EPA Category III.

The example chosen here simply to illustrate the point is one of the more extreme cases but this does demonstrate the importance of understanding variability in the in vivo assays. As such, this does lead to a question on test variability and correct prediction of classification when the in vivo reference standard is subject to such inherent variability.

4. Comparative human data from clinical studies and accidental exposures proposed to support accuracy of LVET are largely with substances that are mild or non-irritating [lines 303-305].

The work of Griffith et al. (1), Cormier et al. (5) and Freeberg et al. (6, 7, 8) discussed above demonstrates that LVET is capable of identifying severe irritants and does so in the experimental setting including for those materials tested that are known to be corrosives/severe irritants in humans.

The purpose of comparing LVET to human data from clinical studies using the same test materials and to human experience data from industrial and consumer accidental exposures was to: 1) understand the predictive capacity of LVET relative to the human response for the consumer product categories involved and 2) determine whether use of LVET provides a conservative evaluation of eye irritation potential that still over-predicts the human response but less so than the Draize test. As such, taking into account both ethical considerations for the conduct of human studies and the nature of the consumer product types involved, it is entirely to be expected that such LVET to human clinical/experience data should have been generated with materials/products that are in the mild-moderate range of irritancy. No other data can be expected here. This does not detract from the wealth of information that can be established from such studies in which mild-moderate irritants have been evaluated in this way. Key conclusions from such studies include the following:

o Draize (100 uL) dosing in the rabbit over-predicted the human response to 100 uL test material.

This was established as early as 1965 and 1969 by Beckley et al. who conducted two in vivo-clinical study

comparisons in which rabbits and humans were exposed to 100 uL of an undiluted dishwashing product in study 1 (11) and a 5% soap solution and undiluted liquid household cleaner in study 2 (12). In both studies, effects in humans were only or primarily conjunctival whereas effects in the rabbit Draize test were more severe (tissue type, severity and persistence of effects).

Freeberg et al. (7) went on the confirm this in an in vivo-clinical study comparison in which four consumer products (100 % liquid fabric softener, 20% liquid shampoo, 10% liquid hand soap and 4% liquid laundry detergent) were tested using LVET (10 uL) and Draize (100 uL) dosing in both rabbits and humans. Effects in humans with Draize (100 uL) dosing were primarily conjunctival and transient whereas effects in rabbits using Draize (100 uL) dosing were more severe (tissue type, severity and persistence of effects).

o LVET (10 uL) dosing in the rabbit over-predicted the human response to 10 uL and 100 uL test material.

This was established in the same in vivo-clinical comparison study conducted by Freeberg et al. (7) as mentioned in the paragraph immediately above. Again effects in humans using LVET (10 uL) or Draize (100 uL) dosing were primarily conjunctival and transient whereas effects in rabbits using LVET (10 uL) dosing were more severe (tissue type, severity and persistence of effects) although less so than with Draize (100 uL) dosing in the rabbit.

Ghassemi et al. (13) went on to confirm this in an in vivo-clinical study comparison in which a liquid household cleaner was tested undiluted in rabbit LVET and in humans using LVET (10 uL) and Draize (100 uL) dosing. Effects in humans were only conjunctival and transient whereas effects in the rabbit LVET were more severe (tissue type, severity and persistence of effects).

o LVET dosing in the rabbit over-predicted the human response using equivalent LVET dosing in humans.

The in vivo-clinical study comparison conducted by Roggeband et al. (14) with two detergent and cleaning products dosed 1 uL of undiluted dishwashing liquid and 3 u

L of undiluted liquid laundry detergent in rabbits and humans. The dosing volume was established based on ethical considerations in a pilot clinical study and then applied to both rabbits and human in the main study. Effects in humans were primarily conjunctival with any corneal effects being minimal and transitory. More severe effects (tissue type, severity and persistence) were observed in the rabbit. For additional perspective, the dishwashing liquid and liquid laundry detergent tested were formulations that were representative of such products in the marketplace at that time. In the EU, both products would be classified as R36 (irritant) based on LVET data.

From all of these studies, irrespective of the classification of the products involved, key conclusions are that: 1) the severity of effects resulting from Draize (100 uL) dosing in the rabbit is greater than that seen with LVET (10 uL) dosing in the rabbit and 2) both LVET (10 uL) and Draize (100 uL) dosing in the rabbit over-predict the human response in terms of ocular tissues involved, severity of effect and persistence of effect, however the degree of over-prediction observed with LVET (10 uL) dosing in the rabbit is less than with Draize (100 uL) dosing in the rabbit.

5. Accidental exposures are not generally considered to be a reliable source of the true ocular hazard potential since such exposures are likely immediately followed by flushing the eyes with large volumes of water and may not represent the most severe lesion that might be produced by such an exposure [lines 461-464]

Human experience from industrial and consumer accidental exposures is an important source of data that can be integrated in a Weight of Evidence approach to establishment of reference standards. It is recognised that human experience data have strengths and limitations and clearly depend of the quality, robustness and amount of data available.

Three studies that compare LVET with such human experience data are cited in the published scientific literature. The first is a study by Freeberg et al. (6) in which parallel Draize-LVET datasets were compared with human experience from industrial accidents/follow-up of consumer accidental exposures for 29 detergent and cleaning products. This was

followed by a second study for 14 detergent and cleaning products and personal care products (8). The formulations that were included in these evaluations were reflective of product formulations in development/marketed to consumers at that time and were identified as mild-moderate irritants on the basis of MAS scores and Time-To-Clear for ocular responses. Both studies were designed with reporting criteria to maximise quality and consistency of data. Such acceptance criteria included having at least two human exposure data points for each accidental exposure and a known Time-to-Clear for resolution of ocular effects. In the first study (6), for a two year period covering 1979-1980 the authors found 284 exposures to 23 undiluted products that met the defined acceptance criteria. In addition, 231 employee accidental exposure reports involving 24 products were available providing an overall total of 515 reports for 29 products. Using the parameter of Time-to-Clear, analysis of the data identified that in the vast majority of cases, ocular effects resolved within 4 days with no reports of permanent eye damage. Correlation of the rabbit Draize and LVET data for the 29 products involved identified that the LVET data whilst still over-predicting the human response was less so than the Draize test. This was confirmed in the follow-up study by Freeberg et al. (8) in which human experience data were collected over 18 months from mid-1983 to end-1984 for 218 accidental exposures for 14 detergent and cleaning products of 7 different types that met acceptance criteria further refined from the first study. In this second study, the longest time for complete recovery after any human exposure incident was 4 days.

More recently, Cormier et al. (15) reported a similar study comparing LVET to human experience from consumer contacts for a total of 24 products from different categories of detergent and cleaning products over the time period of 1895-1992 for which LVET data were also available. The data from this study confirmed the conclusions of the Freeberg et al. studies (6, 8) by identifying that LVET, while still being over-predictive, better predicts the human response from consumer accidental eye exposure to different categories of consumer products.

These studies are combined with data from other human

experience data sources such as those from: 1) national and regional Poison Control Centres (e.g. Soap and Detergent 1974-75 and 1976 Intermountain Regional Poison Control Centre studies, Pittsburgh Poison Control Centre 1986-1990 study); 2) the National Electronic Injury Surveillance System (NEISS) 1980-1991 study) and 3) individual company and industry association co-ordinated post-marketing surveillance data.

All of this adds up to in excess of 30 years of human experience data that exist for types of consumer products supported by LVET. These human experience data demonstrate that human accidental exposures to such consumer products involve primarily conjunctival effects with any corneal effects being minimal and transitory and with full resolution of ocular effects in the vast majority of cases being within just a few days. This is a very substantial database that should form part of the WoE approach that correlates LVET back to the human response from accidental exposure to consumer products

Indeed there is precedence in the field of herbal medicines in the EU for use of such human experience data in a WoE approach.

To promote consumer safety, the European Commission introduced legislation which requires all unlicensed traditional herbal medicinal products intended for human use to be registered (Directive 2004/24/EC) (16). One of the issues with subjecting herbal medicinal products to the same level of regulatory compliance afforded pharmacologically active medicinal products was the recognition that many traditionally used medicinal substances may have limited formal safety and clinical efficacy data associated with their use and little demonstrated by contemporary clinical and toxicological methodologies and practices. Where this has been demonstrated, such products have received medicinal product marketing authorizations. Retrospective imposition of clinical and toxicological requirements on manufacturers of such products would in all probability remove products from the market that have many years of demonstrable safety associated with established use.

To address this, the European Commission decided to create a legislative framework for a pragmatic assessment of

clinical efficacy and safety based on the principles of well-established use. Under Directive 2004/24/EC (16), if the regulatory authorities determine that sufficient product knowledge exists, applications can be made without the usual dossier information on safety and efficacy associated with medicinal products, and is replaced with a bibliographic review and expert reports to prove that the herbal medicinal product (or an equivalent medicinal product) has been in medicinal use as a traditional medicinal product in the European Union for a period of at least thirty years (or 15 years in the EU plus 15 years outside of the EU).

From this is can be concluded that the extensive human experience database which covers more than three decades is a legitimate data source to support use of LVET as an appropriate in vivo reference standard for the domains of applicability for which such retrospective historical and available data exist.

6. Since its original development, proponents of the LVET have suggested that it is a more appropriate in vivo reference test method for comparisons to in vitro data than is the Draize rabbit eye test. This is primarily based on the assertion that the LVET is more representative of the human response to a potential ocular hazard than the Draize test, given that the site (corneal surface) and volume of exposure used in the LVET more closely resemble that of accidental human exposure than does the Draize [lines 400-405].

Dose volume is one of the most influential factors that contributes to over-prediction of the human response by the Draize test reported in the scientific literature. The volume of test material instilled into the lower conjunctival sac of the rabbit in the Draize test is 100 u L. This amount exceeds the volume capacity of the rabbit eye lower conjunctival sac that can maximally hold ~80 uL without blinking (17). When 100 uL of test material are placed in the lower conjunctival sac of the rabbit eye, the excess would be expected to spill from the eye. This is actually what is observed in the experimental situation by investigators conducting the Draize Test (18).

Since the tear volume in both the rabbit and humans is

very similar at approximately 7 uL (19, 20) and the volume capacity of the human eye is 10 uL after blinking (17, 21), this would indicate, from an anatomical/physiological viewpoint, that 10 uL is an appropriate choice of dose volume for the in vivo rabbit test. Taking these anatomical/physiological data into account, it is clear that the 10 uL volume is more than the volume that can be in direct contact with either the rabbit or the human eye i.e. more than the total tear volume.

In terms of understanding the volume of material that can contact the human eye in an accidental exposure, it is reasonable also to take the blink reflex into account. Spontaneous blinking continues throughout the waking state and ensures that the continuously secreted tears are adequately distributed across the exposed ocular surface at all times. In the human, the spontaneous blink rate is about 12-20 per minute (22, 23) and serves to refresh the tear film at each blink. This is much more frequent than the spontaneous blink rate of about 3 blinks per hour in the rabbit (24). Adversive blinking in response to a foreign material contacting the surface of the eye is a natural, involuntary and extremely rapid, reflex response that is accompanied by a reflex secretion of tears. Since, the blink reflex is poorly developed in rabbits and highly developed in man, this contributes to an increased conservatism in an in vivo test such as the rabbit LVET or Draize test.

Furthermore, the importance of dose volume and location have been recognised by international scientific organisations such as the National Academy of Sciences (NAS). In 1977, a National Academy of Sciences/National Research Council (NAS/NRC) committee on toxicology reviewed toxicological testing methods for household products for the Consumer Product Safety Commission (CPSC) (25). Whilst recognising that in vivo eye irritation methods have historically called for instillation of 100 u L (or solid equivalent) of a test material into the eye of the rabbit, they acknowledged that the comparative data from controlled exposures of humans and rabbits available at that time (e.g. Beckley et al. (11, 12) showed the responses of the rabbit eye to be much more severe and long-lasting injuries. They also acknowledged that the amount of material that actually contacts the ocular tissues in most accidents is probably considerably less

than 100 uL. They concluded that: 1) since the amount contacting the eye may be as important as the product composition in determining the ocular response, there seemed to be no basis for using a single arbitrary dose in an eye test and 2) the high dose of test material in the in vivo rabbit eye test may be an important factor in explaining the differences between the excessive responses observed in the Draize test and real-life responses observed in humans following accidental exposures to certain classes of products. Based on their review, the Committee suggested the possibility to include use of lower dose volumes in the in vivo test as a means to diminish the ocular irritancy response in the rabbit test enabling a better correlation to the estimated human accidental eye irritation response (25). The Committee also commented on the location for placement of the test material indicating that the desired dose should be applied to the eye in a manner that reflects the probable route of exposure. They recommended placement of the test material directly onto the cornea to better reflect conditions of accidental human exposure. Finally, the Committee advocated that advantage should be taken of any accidental human eye splashes with chemicals to establish some basis for comparison with animal data.

As such, it is concluded that choice of 10 uL as the dose volume for LVET is supported by anatomical/physiological considerations between rabbits and humans.

Though the Draize test has been used as the regulatory accepted in vivo eye irritation assay for decades and hence also as the only in vivo reference standard against which to validate in vitro eye irritation methods, there are, as with any assay, generally recognized limitations of the Draize test. Scientific publications describe challenges of the Draize test related to variability, subjectivity of scoring and over-prediction of the human response (26, 27, 28, 29, 30, 31). These challenges, added to concerns about animal welfare and a scientific desire to have available eye irritation assays that are based on better understanding of eye injury at the tissue and cellular level, have led researchers to investigate 3Rs alternative methods both in vivo (refinement) and in vitro (replacement) methods. LVET is a 3Rs refinement method.

As such, the SRD comment detailed above that reads “Since

its original development, proponents of the LVET have suggested that it is a more appropriate in vivo reference test method for comparisons to in vitro data than is the Draize rabbit eye test" would perhaps be better reflected as proponents of LVET suggest that based on retrospective historical and available data that this test method is an appropriate in vivo reference standard for the domains of applicability for which the data support its use in a WoE approach.

In conclusion, there is an extensive dataset of historically available LVET data that supports use of such existing LVET data as an appropriate in vivo reference standard against which to compare in vitro assays within the context of the current ICCVAM review "Use of In Vitro Methods in a Tiered Approach for Ocular Hazard Identification of Anti-Microbial Products". Furthermore, it provides data for several characteristics of the assay that are key to scientific acceptance of available LVET data for domains of applicability for which the data support its use as a reference standard in a WoE approach. These are:

- Anatomical and physiological basis for choice of 10 uL as an appropriate dose volume
- Ability of 10 uL dose volume to effectively discriminate between materials of different eye irritancy potential
- Ability of LVET to detect the range of ocular responses from innocuous to severe
- Ability of LVET to correctly predict known severe human eye irritants
- Over-prediction of the human response by LVET, but to a lesser extent than the Draize test, thereby remaining a conservative evaluation of eye irritation potential
- Correlation of LVET dosing procedure with the human response in clinical studies
- Correlation of LVET data and human experience data from industrial accidents and consumer accidental exposure for the same consumer products

To not use this extensive historical database on LVET to accept this assay as an appropriate in vivo reference standard for domains of applicability for which the available data support its use in a WoE approach against which to compare in vitro assays would indeed be a badly

missed opportunity to support progress to validation of in vitro eye irritation assays.

I thank you for the opportunity to make this public comment and ask that it be made available before the independent scientific peer review panel on alternative ocular safety testing methods that will take place on May 19-21, 2009.

Yours sincerely,

Dr. R.A. Rapaport,
Associate Director,
Product Safety & Regulatory Affairs,
The Procter & Gamble Company

References

1. Griffith, J.F., Nixon, G.A., Bruce, R.D., Reer, P.J. and Bannan, E.A. (1980). Dose-response studies with chemical irritants in the albino rabbit eye as a basis for selecting optimum testing conditions for predicting hazard to the human eye. Toxicol. Appl. Pharmacol. 55, 501-513.
2. Grant, W.M. (1974). Toxicology of the eye. 2nd Ed. Charles C. Thomas, Springfield, Illinois.
3. Maurer, J. K., Parker, R.D. and Jester, J.V. (2002). Extent of initial corneal injury as the mechanistic basis for ocular irritation: key findings and recommendations for the development of alternative assays. Reg. Toxic. Pharmac. 36, 106-117.
4. Jester, J.V. (2006). Extent of corneal injury as a biomarker for hazard assessment and the development of alternative models to the Draize rabbit eye test. Cut. and Ocular Toxic. 25 (1), 41-54.
5. Cormier, E.M., Parker, R.D., Henson, C., Cruze, L.W., Merritt, A.K., Bruce, R.D. and Osborne.R. (1996). Determination of the intra- and inter-laboratory reproducibility of the Low Volume Eye Test and its statistical relationship to the Draize test. Reg. Tox. Pharmac. 23, 156-161.
6. Freeberg, F.E., Griffith, J.F., Bruce, R.D., Bay, P.H.S. (1984). Correlation of animal test methods with human experience for household products. J. Toxic. Cut. & Ocular Toxic. 1(3), 53-64.
7. Freeberg, F.E., Nixon, G.A., Reer, P.J., Weaver, J.E., Bruce, R.D., Griffith, J.F. and Sanders III, L.W.

(1986a). Human and rabbit eye responses to chemical insult. Fund. Appl. Toxic. 7, 626-634.
8. Freeberg, F.E., Hooker, D.T. and Griffith, J.F. (1986b). Correlation of animal test methods with human experience for household products: an update. J. Toxic. Cut. & Ocular Toxic. 5 (2), 115-123.
9. Gettings, S.D., Lordo, R.A., Hintze, K.L., Bagley, D.M., Casterton, P.L., Chudkowski, M., Curren, R.D., Demetrulias, J.L., Dipasquale, L.C., Earl, L.K., Feder, P.I., Galli, C.L., Gay, R., Glaza, S.M., Gordon, V.C., Janus, J., Kurtz, P.J., Marenus, K.D., Moral, J., Pape, W.J.W., Renskers, K.J., Rheins, L.A., Roddy, M.T., Rozen, M.G., Tedeschi, J.P. and Zyracki, J. (1996). The CTFA evaluation of alternatives program: an evaluation of potential in vitro alternatives to the Draize primary eye irritation test (Phase III) surfactant-based formulations. Fd Chem. Toxic. 34, 79-117.
10. Gettings, S.D., Lordo, R.A., Feder, P.I. and Hintze, K.L. (1998). A comparison of Low Volume, Draize and in vitro eye irritation test data. III. Surfactant-based formulations. Fd Chem. Toxic. 36, 209-231.
11. Beckley, J.H. (1965). Comparative eye testing: man vs. animal. Toxicol Appl. Pharmac. 7, 93-101.
12. Beckley, J.H. (1969). Use of the rhesus monkey for predicting human response to eye irritants. Toxicol. Appl. Pharmac. 15, 1-9.
13. Ghassemi, A., Sauers, L.H., Bruner, L.H., Reer, P.J., Hall, R.H. (1993). Demonstrating the human safety of a new household cleaning (HSC) product using alternatives to the Draize eye irritation test. Presentation made at the U.S. Society of Toxicology meeting
14. Roggeband, R., York, M., Pericoi, M and Braun, W. (2000). Eye irritation responses in rabbit and man after single applications of equal volumes of undiluted model liquid detergent products. Fd Chem. Toxic. 38, 727-734.
15. Cormier, E.M., Hunter, J.E., Billhimer, W., May, J., Farage, M. (1995). Use of clinical and consumer eye irritation data to evaluate the Low Volume Eye Test. J. Toxicol. Cut. & Ocular Toxicol. 14, 197-205.
16. Medicines Directive 2004/24/EC amended as regards Traditional Herbal Medicinal Products Directive 2001/83/EC on the community code relating to medicinal products for human use.
17. Swanston, D.W. (1985). Assessment of the validity of animal techniques in eye irritation testing. Fd Chem. Toxic. 23 (2), 169-173.

18. Prinsen, M.K. (2006). The Draize test and in vitro alternatives; a left-handed marriage? Toxicology In Vitro. 20 (1), 78-81.
19. Mishima, S., Gasset, A., Klyce, S.D. and Baum, J.L. (1966). Determination of tear volume and tear flow. Invest. Ophthalmol. 5, 264-276
20. Chrai, S., Patton, T.F., Mehta, A., Robinson, J.R. (1973). Lacrimal and Instilled Fluid Dynamics in Rabbit Eyes. J. Pharmacent. Sci. 62, 1112-1121
21. Ehlers, N. (1976). Pharmacology of the conjunctival sac, In Drugs and Ocular Tissues (Second Meeting of the International Society for Eye Research, Jerusalem, 1976), Dickstein, S. Ed., Karger, S.Basel. p.23.
22. Karson, C.N., Berman, K.F., Donnelly, E.F., Mendelson, W.B., Kleinman, J.E. and Wyatt, R.J. (1981). Speaking, thinking and blinking. Psychiatry Res 5:243-246.
23. Bell, G.H., Emslie-Smith, D. and Paterson, C.R. (1976). Chapter 41: Special senses. In: Textbook of Physiology and Biiochemistry. 9th Edition. Churchill Livingstone, Edinburgh. p. 571.
24. Mann, I. and Pullinger, B.D. (1942). A study of mustard gas lesions of the eyes of rabbits and men. Proc. Roy. Soc. Med. 35, 229-244.
25. Committee for the revision of NAS publication No. 1138. (1977).In Principles and Procedures for Evaluating the Toxicity of Household Substances, pp.41-54. National Academy of Sciences, Washington, D.C. 1977.
26. Weil, C.S. and Scala, R.A. (1971). Study of intra- and interlaboratory variability in the results of rabbit eye and skin irritation tests. Toxicol. Appl. Pharmacol. 19, 276-360.
27. York, M. and Steiling, W. (1996). A critical review of the assessment of eye irritation potential using the Draize rabbit eye test. J. Appl. Pharmac. 18, 233-240.
28. Buehler, E.V. (1974). Testing to predict potential ocular hazards of household chemicals. In: Toxicology Annual 1974. Winek, C.L. (Ed.), Marcel Dekker, p. 53.
29. Heywood, R., James, L.W. (1978). Towards objectivity in the assessment of eye irritation. J. Soc. Cosmet. Chem. 29, 25.
30. Jacobs, G., Martens, M., De Beer, J. (1987). Selecting optimal dosage volumes for eye irritation tests in the rabbit. J. Toxicol - Cut. & Ocular Toxicol. 6, 109-116.
31. Daston, G.P., Freeberg, F.E. (1991). Chapter 16: Ocular irritation testing. In: Dermal and Ocular

Toxicology. Fundamentals and Methods. Hobson, D.W. (Ed.),
CRC Press, Boca Raton. pp. 510-539.

Subject: FR Notice Comments - 74FR14556 - Ocular Peer Panel Meeting
Date: Thursday, May 14, 2009 10:47 AM

Below is the result of your feedback form. It was submitted by
() on Thursday, May 14, 2009 at 10:47:02

Comment_date: May 14, 2009

Prefix: Dr.

FirstName: Gerald

LastName: Renner

Degree: PhD

onBehalfOf: yes

Title: Director Science and Research

Department: Science

Company: Colipa, The European Cosmetics Association

Country: Belgium

Phone:

EMail:

Comments: May 14, 2009

William Stokes, D.V.M., D.A.C.L.A.M.
Director NICEATM,
National Toxicology Program,
P.O. Box 12233, MD K2-16
Research Triangle Park, NC 27709

Dear Dr. Stokes,

This public comment is delivered in response to Federal Register Notice Volume 74, Number 60, Pages 14556-14557. It provides some overview comments from the European Cosmetics Association COLIPA on the Background Review Documents (BRDs) published on April 1, 2009 indicates

COLIPA's intention to be present at the public meeting of the peer review panel meeting to be held on May 19-21, 2009.

COLIPA very much welcomes this activity of ICCVAM to address the Validation Status of Alternative Ocular Safety Testing Methods and Approaches.

As you are aware, COLIPA has been and remains very active in the area of eye irritation alternatives. Our goal is the development and validation of in vitro methods that are more predictive of the human response through better understanding of chemically induced mechanisms of eye irritation. Our overall programme focuses on: 1) development/optimisation of in vitro methods for validation and 2) research on identification and integration of evaluation endpoints based on mechanistic understanding into existing/new in vitro test methods. In light of this, we would like to offer the following general overview comments:

- We acknowledge that replacement of the in vivo test will require combinations of in vitro assays. We would welcome discussion on the possibility of statistical approaches that will be necessary to allow decision making from complex matrices of data on individual in vitro assays and their domains of applicability in a tiered testing strategy.

- We would encourage primary use of specific domains of applicability to define the acceptability of an in vitro assay to predict a defined level of eye irritation. This would favour more correct prediction of classification using combinations of in vitro assays in a tiered testing strategy.

- We would welcome discussion on use of a Weight of Evidence (WoE) approach to identify the in vivo reference standard against which to validate in vitro test methods. This would include discussion of the role of human experience data from Poison Control Centres and industry (cosmeto/pharmacoviligance) systems. Data from these sources can span more than four decades.

- We are presented with an important opportunity to use a WoE approach to further retrospective analysis to

validate alternative methods/strategies for eye irritation and identify future research and validation needs.

- Such retrospective analysis would allow us to identify further research needs on mechanisms of chemically induced eye irritation e.g. physiological mechanisms involved in reversible injury which are key to prediction of eye corrosives and severe eye irritants.

- We would welcome further discussion on harmonisation of approaches/activities for retrospective validation of in vitro assays for eye irritation in the context of the recently established International Cooperation on Alternative Test Methods (ICATM).

Subject: FR Notice Comments - 74FR14556 - Ocular Peer Panel Meeting
Date: Friday, May 15, 2009 2:42 PM

Below is the result of your feedback form. It was submitted by
() on Friday, May 15, 2009 at 14:42:58

Comment_date: May 15, 2009

Prefix: Dr.

FirstName: Sherry

LastName: Ward

Degree: PhD, MBA

onBehalfOf: no

Title:

Department:

Company:

Country: USA

Phone:

EMail:

Comments: Where are the stratified human corneal epithelial cell models?

The following ATLA article provides a good overview of many of the ocular methods being reviewed this year by ICCVAM and ECVAM:
Eskes, et al. (2005). Eye irritation. Altern. Lab. Anim. [ATLA] 33, Suppl. 1, 47-81.

If you take the time to browse through this ATLA article, the one method you will notice that is missing from the 2009 Ocular Panel review is the Gillette HCE-T Model (page 57).

I'd like to take this opportunity to correct a number of

errors present in the 2005 ATLA article section that describes the HCE-T model (page 57), and to provide some references for those who would like to learn more about the performance of that method for ocular toxicity testing.

1) Key references were omitted from that review, and are provided below.

2) The prevalidation study involved testing only surfactant-containing formulations, however, the 4-lab validation study included both surfactant formulations and surfactants. A summary of the prevalidation study results was published along with a detailed description of the mechanistic basis of the test method and the biological relevance of the model (Ward, et al., 2003). The validation study results were written up in the form of a Background Review Document, but the company decided to not submit or publish the results.

3) Fields of application: The validation study focused on surfactant formulations and surfactants. A previous publication (Kruszewski, et al., 1997) provided the results of testing other chemical classes using this test method.

Prior to the conduct of the validation study, cationic surfactants were identified as incompatible with the fluorescein permeability (TEP) assay, due to the mechanism of action of that kind of surfactant on the cells. Cationics fix the cells in place, but the cells are dead and permeable and therefore take up the fluorescein. This prevents a quantitative leakage of fluorescein through the cell layers into the basal chamber. Other cytotoxicity assays are compatible with the HCE-T model (MTT, lactate, etc.), and can be used for testing cationic surfactants.

4) The HCE-T TEP method was useful for determining the ocular toxicity of substances across the range of in vivo ocular irritation, but may not have been sufficiently evaluated with severe materials which must be tested in diluted form when used in the 5 minute exposure protocol.

5) The method was extremely sensitive, and substances causing slight differences in degree of irritation could be reproducibly distinguished. A different assay

(transepithelial electrical resistance, TER) which evaluates disruption to the surface cell tight junctions was an even more sensitive indicator of ocular injury.

A battery of 3 endpoints was evaluated for a limited number of materials, and found to be even more predictive of the Draize score than the TEP data alone.

The ATLA article says that “histomorphology can also be used as an endpoint.” In my opinion, histomorphology was very useful in understanding the mechanism of action of chemicals on the cells; I would not use it as an endpoint.

6) On-going developments: None known, although the cells are available from the ATCC. ATCC reports for many years indicated that many companies and academic labs purchased and used the cells for research and internal testing applications.

The membrane/culture insert used during these studies may no longer be available. Data developed before this membrane was selected for the HCE-T model showed that the cells grew and stratified equally well on several other commercially-available inserts (and poorly on some).

7) INCORRECT last statement in ATLA article: The validation study was NOT restricted to surfactant-containing formulations. Both the prediction model and the test materials consisted of surfactants and surfactant formulations. A major reason for limiting the study to these types of materials was the difficulty in getting a sufficient number of other types of test materials with quality in vivo data for the study. The error in this last statement is surprising considering that 3 of the authors on this paper were associated with and had direct access to all of the validation study documents and data.

Summary:
Newer versions of stratified human corneal epithelial cell models have been developed. They probably share many or all of the same characteristics as the HCE-T model, so the data and experience from prior studies using this model should be useful in guiding new validation studies.

Key HCE-T References:

Ward, S.L. Gacula Jr., M., and Edelhauser, H.F. (2003). The Human Corneal Epithelial HCE-T TEP Assay for Eye Irritation: Scientific Relevance and Summary of Prevalidation Study Results. In: Alternative Toxicological Methods for the New Millennium. (Eds. H. Salem & S.A. Katz). CRC Press, Boca Raton, FL. pp. 161-186.

Clothier, R., Orme, A., Walker, T.L., Ward, S.L., Kruszewski, F.H., DiPasquale, L.C., and Broadhead, C.L. (2000). A comparison of three cytotoxicity assays using the corneal HCE-T model. Altern. Lab Anim. 28, 293-302.

Kruszewski, F.H., Walker, T.L. and DiPasquale, L.C. (1997). Evaluation of a human corneal epithelial cell line as an in vitro model for predicting ocular irritation. Fundam. Appl. Toxicol. 36:130-140.

Ward, S.L., Walker, T.L., and Dimitrijevich, S.D. (1997). Evaluation of chemically-induced toxicity using an in vitro model of human corneal epithelium. Toxicol. In Vitro. 11, 121-39.

Ward, S.L. (1996). Research needs for the development of improved alternatives to the Draize eye test. In “Replacing the Draize Eye Irritation Test: Scientific Background and Research Needs” by the ILSI Health and Environmental Sciences Institute Technical Committee on Alternatives to Animal Testing. J. Toxicol. Cut. Ocul. Toxicol. 15, 224-29.

Kruszewski, F.H., Walker, T.L., Ward, S.L., and DiPasquale, L.C. (1995). Progress in the use of human ocular tissues for in vitro alternative methods. Comments on Toxicol. 5, 203-24.

Kahn, C.R., Young, E., Lee, I.H. & Rhim, J.S. (1993). Human corneal epithelial primary cultures and cell lines with extended life span: In vitro model for ocular studies. Invest. Ophthalmol. Vis. Sci. 34:3429-3441.

Documents submitted to NICEATM-ICCVAM:

Gillette 12/6/99 BRD (1999). Prevalidation / Validation Study for the HCE-T TEP assay (Pre-study plan).

Gillette 5/11/00 Report (2000). Responses to comments and

questions from the ICCVAM Ocular Toxicity Working Group, and modifications to the December 6, 1999 Background Review Document "Prevalidation / Validation Study for the HCE-T TEP Assay."

Gillette 1/29/01 BRD. (2001). Prevalidation Study Results for the HCE-T TEP Assay Background Review Document.

References for related human conjunctival epithelial cell model:

Smit, E.E., Sra, S.K., Grabowski, L.R., Ward, S.L., and Trocme, S.D. (2003). Modulation of IL-8 and RANTES release in human conjunctival epithelial cells: Primary cells and cell line compared and contrasted. Cornea 22, 332-337.

Ward, S., Walker, T., Trocme, S., Hallberg, C., Kruszewski, F., and DiPasquale, L. (2000). A human conjunctival model for the evaluation of eye irritants. In: Progress in the Reduction, Refinement and Replacement of Animal Experimentation. (Eds: M. Balls, A.-M. van Zeller, and M.E. Halder). Elsevier Science B.V., Amsterdam.

June 22, 2009

Chair and Members
NTP Scientific Advisory Committee on
Alternative Toxicological Methods (SACATM)
National Institute of Environmental Health Sciences
P.O. Box 12233, MD EC-17
Research Triangle Park, NC 27709

Re: Pilot EPA/OPP Antimicrobial Cleaning Product Labeling Program *vώ-à-vώ* Report of the Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods

Dear Dr. Freeman and SACATM Members:

These comments are submitted on behalf of The Humane Society of the United States, Humane Society Legislative Fund, People for the Ethical Treatment of Animals and Physicians Committee for Responsible Medicine. The parties to this submission are national animal protection scientific and public interest organizations with a combined membership of more than 12 million Americans, and longtime stakeholders in the 3Rs (replacement, reduction and refinement) efforts of US federal agencies and the interagency entities.

We wish to advise the Committee of our strong support for a recent initiative by the US Environmental Protection Agency's Office of Pesticide Programs (EPA/OPP) to implement a pilot eye irritation labeling program for antimicrobial cleaning products. As the federal agency responsible for establishing and implementing a regulatory scheme for the hazard labeling of pesticide products, it is fully within EPA/OPP's purview to determine whether a test method or strategy is valid for a particular use within the scope of its regulatory activities. Such a decision is made especially straightforward when a method or strategy has been subject to the degree of validation to which the antimicrobials ocular labeling strategy has undergone.

With this in mind, we respectfully encourage other US agencies to heed EPA/OPP's example by reserving ICCVAM reviews for tests/strategies with multi-agency applicability, as well as by adopting a streamlined approach to agency acceptance of methods/strategies deemed scientifically valid in other regions of the world.

Sincerely,

[Redacted]

Troy Seidle
Director of Science Policy
The Humane Society of the United States
[Redacted]

Sara Amundson
Executive Director
Humane Society Legislative Fund

[Redacted]

Martin Stephens, PhD
Vice President, Animal Research Issues
The Humane Society of the United States

[Redacted]

Kate Willett, PhD
Science Policy Advisor
People for the Ethical Treatment of Animals

[Redacted]

Chad Sandusky, PhD
Director of Toxicology & Research
Physicians Committee for Responsible Medicine

cc: Dr. L. Birnbaum
Dr. D. Edwards
Dr. T. Levine
Dr. J. Fowle

June 25, 2009

Dr Mary S. Wolfe
National Institute of Environmental Health Sciences
PO Box 12233, MD A3-01
Research Triangle Park, NC 27709

PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

HEADQUARTERS
501 FRONT ST.
NORFOLK, VA 23510
757-622-PETA
757-622-0457 (FAX)

Re: **74 FR 19562; April 29, 2009; National Toxicology Program (NTP); Office of Liaison, Policy and Review; Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)**

Dear Dr Wolfe:

People for the Ethical Treatment of Animals (PETA) is the world's largest animal rights organization, with 1.7 million members and supporters. We appreciate the continued opportunity to comment regarding the NICEATM/ICCVAM 5-Year Plan, in this instance in commenting on the *Draft Implementation Plan* (hereafter referred to as the "Draft Plan") by presenting oral comments at the meeting of SACATM June 25, 2009, and request the opportunity to submit formal written comments on the Draft Plan itself.

General Comments

We continue to believe that ICCVAM should focus its limited resources on methods that have applicability to more than one member agency.

We are encouraged to see that ICCVAM has created a Research and Development Working Group (RDWG) whose task is to help NICEATM/ICCVAM identify and promote research that incorporates new technologies. We and others, including SACATM's internal review committee for the 5-year plan, strongly advocated for a pro-active element to ICCVAM for bringing developing methods to the table for further development and validation. We will be anxious to learn the specifics about this committee including, for example, who the members of this committee, how they were selected, and a detailed plan for future activities.

For several of the Priority Areas, sections titled "Specific Objectives" and "Planned Activities for Implementation contain generic descriptions. It would be helpful if these sections contained some detail and context for the planned work, for example a summary of the state-of-the-art, how the planned activities will build on the existing foundation, and a description of the intended outcome of the activities. Similarly, descriptions of "Accomplishments" list past activities, such as "a peer review panel met" and "a report detailing the conclusions and recommendations resulting form this workshop is available." It would again be helpful if a summary of the outcome and conclusions of these activities was given, so that progress within a given area could be tracked.

A general comment about the envisioned workshops: ICCVAM workshops, both in the past and planned, regardless of topic, have the same generic goals and consist of the same generic elements. This organization does not inspire confidence that progress will be achieved; in fact,

as a case in point, these same goals and elements were used in the Workshop on Acute Chemical Testing: Advancing *In Vitro* Approaches and Humane Endpoints for Systemic Toxicity Evaluations, Feb 6 – 7, 2008. In this workshop, there was no discussion of the results of previous ICCVAM (or other) workshops on the same topic, no presentation of previous or ongoing work on the subjects, and no context for the questions asked. As a result the discussions were repetitive and did not substantially further the discussion topics. A more effective approach would be to tailor each workshop to the subject, beginning with the current state-of-the-art, inviting relevant experts that are at the forefront of the respective topics to be covered, and formulating discussion topics with defined goals in mind. Such an approach should be applied to the many workshops proposed in the Draft Plan.

General comments about Peer Reviews: Observers of two recent peer reviews, a review of Five *In Vitro* Test Methods Proposed for Assessing Potential Pyrogenicity of Pharmaceuticals and Other Products in February, 2007, and most recently an Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Strategies in May, 2009 (described in detail below), noted some similar procedural difficulties. In both cases, it was evident from the Peer Review Panel (PRP) discussion that the panel did not have a comprehensive view of the subject it was reviewing and apparently misunderstood its charge. In both cases, panel members appeared unaware of the validation and acceptance procedures, the PRP's role, or the ICCVAM process. It appeared as though panel members were provided no background information on the state-of-the-art of the current procedures and methods; panel members appeared to have unreasonable expectations regarding details of the alternative methods without a clear understanding of the limitations of the current animal-based tests. In addition, experts and stakeholders that were not allowed to interact with the panel, and were only allowed to comment ***after*** the panel had deliberated and made its recommendations. Panel members were not aware that they could ask questions of the experts present.

The peer review process could be greatly improved by providing the panel with appropriate background and context for review, along with a simple sent of focused questions for the review.

A note on expedited review (lesson learned from pyrogenicity): The European Center for the Validation of Alternative Methods (ECVAM) nominated five *in vitro* pyrogenicity methods to ICCVAM in June of 2005. Following an additional extensive and lengthy review that included a full peer review, ICCVAM issued its final recommendations in November 2008. A comparison of the letters written by then-Acting Director Wilson to US federal agencies[vii] and the ECVAM Scientific Advisory Committee statement, published in March of 2006,[viii] reveals the conclusions of each committee to be nearly identical. Delays such as this are a waste of precious time and resources. A much more expedited process is needed for reviewing methods that have already undergone extensive peer reviews.

Specific Comments

Challenge #1: Conduct and Facilitate Alternative Test Method activities in Priority Areas

Biologics Testing

The Goal and Specific Objectives in this section lack sufficient description to evaluate; however, there are a number of initiatives in this area that ICCVAM should take into account and build from when planning its activities, particularly the workshop mentioned.[123] Specifically, note should be taken of the progress made by the European Centre for the Validation of Alternative Methods (ECVAM) to validate the following: ELISA test for batch potency testing of tetanus vaccines for human use, Toxin Binding Inhibition (ToBI) test for batch potency testing of tetanus vaccines for human use, and ELISA test for batch potency testing of erysipelas veterinary vaccines, Newcastle Disease Virus (NDV) for veterinary use.

In addition to implementing the ECVAM-validated methods, the European Directorate for the Quality of Medicines and HealthCare (EDQM) is seeking to make progress on the following vaccine potency tests: Pertussis, Tetanus, Diptheria, HepA, HepB, HPV-VLP, smallpox, Yellow Fever, IPV, TBE, among others. If ICCVAM were to hasten the replacement of animal-based potency tests on these as well as other vaccine potency tests in the U.S., a great deal of animal testing would be avoided.

EDQM has also made allowances for companies to avoid target-animal safety test (TAST) for batch safety testing of vaccines for veterinary use after an appropriate number of safety tests have been completed for consecutive batches. The elimination of the target animal safety test for vaccine safety testing in the U.S. would harmonize with EU regulations thus allowing for a greater number of animal tests that would be avoided.

Leptospirosis: It is not clear what the need is for ICCVAM review of Leptospirosis vaccine potency tests being used by the USDA; these methods have been deemed appropriate and are already in use by the USDA, and there is no other agency need for these tests. USDA Supplemental Assay Methods (SAM) 624, 625, 626, and 627 allow for the use of the sandwich ELISA method for serovars *pomona, canicola, grippotyphosa,* and *icterohaemorragiae* for *Leptospira interrogans* vaccines. The successful implementation of these analytical methods (in lieu of the hamster test) has been verified by USDA as well as the pharmaceutical industry.[456]

Ocular Toxicity Testing

As an accomplishment of 2009, ICCVAM describes the Independent Scientific Peer Review Panel Meeting: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Strategies, which was held May 19- 21, 2009. This peer review was ostensibly in response to the submission of an approach to assess ocular irritation by a consortium of manufacturers of

---

[1] Hendriksen, C. Refinement, Reduction, and Replacement of Animal Use for Regulatory Testing: Current Best Scientific Practices for the Evaluation of Safety and Potency of Biologicals. 2002. ILAR Journal (43) S43-S48.
[2] Cussler, K. et al. Humane Endpoints in Vaccine Research and Quality Control. 2002. Altern. Lab Anim. 30(1):93-108
[3] Halder, M. et al. *ECVAM's Activities on Biologicals*. 2002. ATLA 30:125-128.
[4] United States Department of Agriculture Center for Veterinary Biologics Testing Protocol (SAM 624) Supplemental Assay Method for *in vitro* Potency Testing *of Leptospira interrogans* Serovar *pomona* Bacterins
[5] United States Department of Agriculture Center for Veterinary Biologics Testing Protocol (SAM 625) Supplemental Assay Method for *in vitro* Potency Testing *of Leptospira interrogans* Serovar *canicola* Bacterins
[6] United States Department of Agriculture Center for Veterinary Biologics Testing Protocol (SAM 627) Supplemental Assay Method for *in vitro* Potency Testing *of Leptospira interrogans* Serovar *icterohaemorrhagiae* Bacterins

antimicrobial cleaning products (AMCP) and the Institute for In Vitro Sciences (IIVS). The consortium had been working for several years to develop and evaluate a completely non-animal method to assign ocular hazard categories required for EPA registration of AMCPs and the consortium kept ICCVAM apprised of its activities from very early in the process.

1. ICCVAM had been asked by EPA and the consortium to assess the general question of whether the proposed testing strategy would "assure EPA, with a reasonable degree certainty, that the Agency can make labeling decisions for antimicrobial cleaning products that appropriately inform the user?"
    a. ICCVAM had agreed to an expedited review; the extensive peer review therefore came as a surprise to the consortium.
    b. ICCVAM did not contact any of the participants in the consortium's effort to present the logic behind the proposal to the Peer Review Panel.
2. As part of the review, ICCVAM took it upon itself to review the validation status of the "low volume eye test" (LVET) method, which is a refinement of the Draize rabbit test and is a method that provided some of the data for the consortium's validation studies.
    a. The request additional review was unexplained since:
    b. European Centre for Validation of Alternative Methods (ECVAM) is currently reviewing this method
    c. ECVAM has compiled a comprehensive Background Review Document
    d. Only a subset of the data available to ECVAM is available to ICCVAM.
    e. The Draize test is known to significantly over predict the human response therefore
    f. the LVET method was specifically designed to be less sensitive that the traditional Draize test and more predictive of humans.
3. The ICCVAM peer review panel concluded that it was necessary to change the scoring system of the LVET to replicate exactly the Draize results.
    a. The Panel recommended a full validation study be done using approximately 50 chemicals to compare the LVET with the traditional Draize,
    b. enough data already exists to compare the two methods. In addition, the Consortium provided both animal and *in vitro* data on more than 60 antimicrobial (or similar) cleaning products (which represent the major proportion of all AMCPs on the market) yet the Panel concluded that there were not enough data to make a determination.

It was evident from the Peer Review Panel discussion that the panel did not have a comprehensive view of the subject it was reviewing and apparently misunderstood its charge[7]. The stakeholders that were present, including representatives from participants in the consortium, were only allowed to comment ***after*** the panel had finished its discussion and made its recommendations. The Panel itself was not instructed that it could ask questions of the consortium members; therefore any real debate or discussion was prohibited between consortium members and the Panel.

In the meantime, due to the lack of progress of ICCVAM on this topic, the EPA has independently initiated a pilot program which will allow, under certain conditions, for the proposed non-animal testing strategy to be used to register AMCP with the EPA.

[7] This is a continual concern within the ICCVAM process, and it has been raised by us on at least two other occasions, most notably with regard to the ICCVAM review of five *in vitro* pyrogenicity methods in February 2007. See below.

While we also applaud the use of NIH Small Business Innovation Research (SBIR) grants to fund the development and validation of non-animal methods, we question the appropriateness of the use of the SBIR mechanism for the particular topics mentioned here: the use of an alternative corneal holder and the effect of modifying test method components on accuracy and/or reliability. These topics have been in the ICCVAM plan for years and are relatively simple straightforward assessments, yet are proposed for SBIR initiatives in 2009/2010, in which case no work would actually be done until 2011/2012 at the earliest.

Acute Toxicity Testing

The first three Specific Objectives listed in this section are the same objectives that were to have been addressed in the previous two ICCVAM workshops. ICCVAM first considered *in vitro* methods for estimating actuate toxicity in 2000. Following an initial workshop, ICCVAM published a report suggesting follow-up: "Continued development and optimization of such systems (as gut absorption, BBB passage, key kinetic parameters, and metabolism) for this application should be encouraged and should receive regulatory support" as well as concluding "…if the commitment to conducting a formal validation study was strong enough, the scientific resources could be harnessed for this effort with facility and the *in vitro* tests studied proved good enough, a replacement test battery might be achieved in as short a time as 2-3 years."[8] To the best of our knowledge, none of the suggestions have been taken up. In 2008, ICCVAM finally issued recommendations to agencies that cytotoxicity methods could be used to ***set starting doses*** for acute toxicity testing. As listed as an accomplishment for 2008, ICCVAM held a second workshop addressing these same issues. In spite of these workshops, ICCVAM has made no progress toward replacing the use of animals in acute toxicity testing since it began working on this issue in 2000.

Under "Planned Activities for Implementation," the second point, work with stakeholders to promoted the collection and submission of *in vitro* and *in vivo* data in order to "advance the development and validation" of more predicitive *in vitro* test methods and more humane endpoints is too vague to be evaluated as a plan. What, exactly, is to be done, and how will it be accomplished? It is not clear how the third point, namely, participation in a group evaluating biotransformation using human cells, will accomplish any of the Specific Objectives listed in this section.

Endocrine Disruptors Testing

One of the stated purposes for creation of ICCVAM was to validate methods for the EPA's Endocrine Disruptor Screening Program (EDSP), yet not a single assay that is currently on the EDSP list has been evaluated by ICCVAM. While it is a laudable goal for ICCVAM to review and validate appropriate assays, ICCVAM's inaction in this area has driven the EPA to conduct its own validation exercises for methods not validated by Organisation for Economic Co-operation and Development Test Guidelines Programme (OECD) to be included in the Tier 1 battery of the Endocrine Disrupter Screening Program (EDSP).[9]

[8] National Institutes of Health. 2001. Report of the International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity. NIH Publication No: 01-4499 (http://iccvam.niehs.nih.gov/docs/acutetox_docs/finalrpt/finalall0801.pdf)

[9] For a summary of test method validation and links to Peer Review Reports, see: http://www.epa.gov/endo/pubs/assayvalidation/status.htm (accessed 23 June, 2009).

Meanwhile, ICCVAM's review of the LumiCell estrogen receptor bioassay, which began over four years ago, has not been completed and the EDSP is continuing without this assay or the CertiChem, Inc., MCF-7 cell proliferation assay that is also under review by ICCVAM.[10]

Challenge #2: Incorporating New Science and Technology

Nanomaterials Testing

Again, the workshop plan description is too generic to evaluate; however, any workshop in this area should take into account and invite participants from the large international efforts already underway. A positive element of the plan for the one-day symposium to define activities within ICCVAM agencies is the explicit request for agencies to "identify current of new members with expertise specific to nanomaterials" to participate in the workshop. It would be beneficial to include such criteria in all ICCVAM activities.

High Throughput Screening

While the first part of the Specific Objective, "Facilitate the review of the usefulness and limitations of defined HTS approaches" would seem to be an appropriate action for ICCVAM, it is not clear what ICCVAM intends by the second part: "and also assist in the identification of assays and endpoints that are relevant for alternative test methods that have already been adopted." It is also not clear how the planned activates relate to or will accomplish the Specific Objective. A major issue for the incorporation of HTS data in the regulatory process is to define when and were the data can be applied; this would involve detailed conversations with regulators akin to those initiated at the recent NAS Symposium on Toxicity Pathway-Based Risk Assessment: Preparing for Paradigm Change, held in Washington, DC on May 11 – 13, 2009 (which was attended by members of NTP but not of ICCVAM).

Furthermore, evaluation of HTS and other battery approaches (such as the EDSP Tier 1 screening battery) is likely to require a different assessment paradigm than ICCVAM has developed for assessing individual tests; if ICCVAM is to be prepared to evaluate this rapidly evolving technology, this Implementation Plan should articulate the development of such an assessment strategy.

Challenge #3: Fostering acceptance and Appropriate Use of Alternative Test Methods

NICETAM-ICCVAM Website

The current version of the website is quite an improvement in terms of ease of navigation and access to documents and timelines. A significant contribution to the use of the information contained within ICCVAM's documents would be to extract the data into searchable data bases like the ToxRefDB (or perhaps even incorporate the data into this NTP database). ICCVAM's website should also inter-link with the extensive website on implementation of the NRC's Toxicity Testing

[10] Environmental Protection Agency. Agency Information Collection Activities; Submission To OMB for Review and Approval; Comment Request; Tier 1 Screening of Certain Chemicals Under the Endocrine Disruptor Screening Program (EDSP); EPA ICR No. 2249.01, OMB Control No. 2070-New [Federal Register Notice: April 15, 2009 (Volume 74, Number 71, pages 17477-17479)]

for the 21st Century currently being constructed by the EPA's Pesticide Program Dialogue Committee.

Not mentioned: A prominent role for ICCVAM could be to facilitate the use of alternative methods within agencies via its members; agency representatives should have the ability to ensure implementation of ICCVAM-recommended methods within their agency.

Challenge #4: Developing Partnerships and Strengthening Interactions with ECCVAM Stakeholders

As no specifics are presented in this section, the same comments we provided for the Five-year Plan itself are appropriate for this section of the Implementation Plan:

> "This Chapter represents yet another missed opportunity. The draft Plan contains only descriptions of past approaches to developing partnerships and fostering interactions, with several promises to continue these same approaches, all of which again which have achieved very limited success over the past decade. The point of requesting a 5 year plan is to *re-strategize*, to develop *new* approaches to *improve* and *strengthen* interactions. Again, several suggestions were provided in the animal protection community's December 2006 comments, none of which have been incorporated into the draft Plan."

In conclusion, we hope ICCVAM will build on the suggestions contained in these comments to provide a more concrete and detailed implementation plan for its next five years. In addition, we also hope there will be an opportunity to submit formal comments on the Draft Implementation Plan.

Sincerely,

[Redacted]

Catherine Willett, PhD
Science Policy Advisor
Regulatory Testing Division
Research and Investigations Department
People for the Ethical Treatment of Animals
501 Front Street, Norfolk, VA 23510
Tel/FAX: 617-522-3487

[vii] Letter from Samuel Wilson to Elias Zerhouni. Dated 23 October 2008. Available at: http://iccvam.niehs.nih.gov/methods/pyrogen/transmitNov08/ZerhouniLtrPyroF.pdf; Accessed 12 December 2008.
[viii] ESAC Statement on the Validity of In-Vitro Pyrogen Tests. Published 21 March 2008. Available at: http://ecvam.jrc.it/. Accessed 16 December 2008.

This page intentionally left blank

Appendix E3

Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) Comments

SACATM Meeting on June 25-26, 2009

The following is excerpted from the final minutes and speaker presentations of the SACATM meeting convened on June 25-26, 2009. The full meeting minutes are available online at: http://ntp.niehs.nih.gov/go/8202

This page intentionally left blank

Minutes from the June 25 – 26, 2009 SACATM Meeting

X. Report on the Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods

A. Presentations

Dr. Merrill, FDA, presented an introduction and overview of the proposed methods and approaches. She explained the public health importance of ocular safety testing and hazard labeling and said that 15% of all eye injuries are due to chemicals. The Draize Rabbit Eye Test, which involves instillation of 100 μL (liquids) or 100 mg (solids) of a

test substance into the lower conjunctival sac of one eye of a rabbit, is the *in vivo* test method currently accepted by US Federal and international regulatory agencies.

The Ocular Peer Review Panel, "the Panel," met on May 19 -21, 2009; their report will be available in July. ICCVAM plans to transmit recommendations to Federal agencies in December and request responses by June 2010.

The Panel evaluated:

- Routine use of topical anesthetics, systemic analgesics, and humane endpoints to avoid or minimize pain and distress during *in vivo* ocular irritation testing
- Validation status of four *in vitro* test methods for identifying mild/moderate ocular irritants and substances not labeled as irritants: BCOP, ICE, HET-CAM, and IRE
- Validation status of the *in vivo* low volume eye test (LVET)
- Validation status of the individual test methods and testing strategies to assess eye irritation potential of AMCPs, including use of the BCOP, Cytosensor Microphysiometer® (CM), and EpiOcular™ (EO) test methods

Dr. Merrill briefly reviewed the procedures for conducting the test methods, summarized the test method data, and then presented ICCVAM's draft proposed recommendations for their use and limitations. She then summarized the ICCVAM charges to the Panel and acknowledged ICCVAM and the ICCVAM Ocular Toxicity Working Group.

Dr. A. Wallace Hayes, Harvard School of Public Health and Peer Panel Chair, presented a summary of the Panel report. The Panel was composed of 22 members from six different countries and they came to complete consensus on all but one of the recommendations (see HET-CAM below). He acknowledged the support of NICEATM and in particular, the contract support staff. He detailed the ICCVAM charges to the Panel and summarized the Panel's recommendations:

- The Panel proposed an alternative preemptive pain management protocol that should be used for all *in vivo* rabbit eye irritation tests intended for regulatory safety testing, unless there is requirement for monitoring the pain response.
- The Panel concluded that, based on the available data and information, some humane endpoints recommended by ICCVAM are adequate to terminate a study.
- The Panel supported the ICCVAM draft recommendation that the available data and ICE test method performance do not support its use to identify substances from all hazard categories as defined by Globally Harmonized System (GHS), EPA, and EU classification systems.
- The Panel agreed with the ICCVAM draft recommendation that the available data and ICE test method performance do not support its use as a screening test to identify substances not labeled as irritants from all other hazard categories as defined by GHS, EPA, and EU classification systems.

- The Panel supported the ICCVAM draft recommendation that the available data and BCOP test method performance do not support its use to identify substances from all hazard categories as defined by GHS, EPA, and EU classification systems.
- The Panel agreed with the ICCVAM draft recommendation that the available data and BCOP test method performance support its use as a screening test to identify substances not labeled as irritants when results are used for EU or GHS hazard classifications.
- The Panel concluded that the BCOP test method cannot be used as a screening test to identify EPA Category IV substances.
- The Panel supported the ICCVAM draft recommendation that the available data and HET-CAM test method performance do not support its use to identify substances from all hazard categories as defined by GHS, EPA, and EU classification systems.
- The Panel (with one minority opinion) did not support the ICCVAM draft recommendation that the available data and HET-CAM test method performance support its use as a screening test to identify substances not labeled as irritants when results are used for EU or GHS hazard classifications.
- The Panel concluded that additional optimization and validation studies that include all four recommended endpoints are needed before definitive recommendations on the relevance and reliability of the IRE test method can be made.
- The Panel concluded that in the absence of all data, including the ECVAM BRD, they could not make definitive conclusions or recommendations on the validation status of the LVET.
- The Panel agreed with the ICCVAM draft recommendation that the CM test method can be used as a screening test to identify both ocular corrosive/severe irritants and substances not labeled as irritants in a tiered-testing strategy, as part of a weight-of-evidence approach, but this use is limited to surfactant chemicals and specific types of surfactant-containing formulations (e.g., cosmetics and personal care products).
- The Panel agreed with the ICCVAM draft recommendation that there were insufficient data to support use of the AMCPs testing strategy (i.e., using the BCOP, CM, and EO test methods) for classification of substances in all four ocular hazard categories.
- The Panel agreed with the ICCVAM draft recommendation that there were insufficient available data on which to base definitive recommendations on the proposed alternate testing strategy (i.e., using the BCOP and EpiOcular™ test methods) for classifying substances in all four ocular hazard categories.
- The Panel recognized that the use of histopathological evaluation as an additional endpoint does not improve the accuracy and predictability of the BCOP test method for the limited database of currently tested AMCPs; however, histopathological evaluation may prove to be a useful endpoint and as such, collection of ocular tissue and further efforts to optimize histopathological evaluation is strongly encouraged.

Dr. Levine said she saw nothing in the flow chart that required all three tests to be used at the same time. Dr. Hayes said that the concern of the Panel was that it would have been very helpful to know comparative results of compounds tested in all three tests to allow them to adequately evaluate the overall performance of the proposed testing strategy.

B. Public Comments

Dr. Rodger Curren, IIVS, asked the attendees to read the written comments he would be sending for posting on the Website. He addressed Dr. Levine's comment regarding materials not being tested in all three assays and said many antimicrobials were tested in each of the assay systems. Twenty-eight materials were fully evaluated in all the tests and there were no differences in results among the tests. He could understand if there were considerable differences in the chemistry of the materials, then testing in all three assays might be needed, but otherwise it was not. He suggested a way to strengthen the peer review process for additional studies going forward and to improve the efficiency of the reviews. He said there was no effective way for the proponents of an assay strategy or the developers of a new assay to interact with the Panel. Many questions arose in this and other reviews that could have been answered quickly by the writers of the BRD or the developers of the assay. The proponents of the assay were allowed to speak only to the methodologies and not to the interpretation. He was not proposing extended debate in the peer review process, but only some way to allow greater interaction with the Panel.

Dr. Kate Willett, PETA, expressed puzzlement that the Panel's evaluation involved such an enormous review when the original nomination was simply for the antimicrobial project.

Dr. Levine asked if EPA's specific charge to ICCVAM, regarding a review of the flow chart's use for making labeling decisions on AMCPs, was communicated to the Panel. Dr. Stokes said the charge was clearly communicated. He further added that bringing a peer review Panel together is very expensive and time-consuming process; therefore, NICEATM-ICCVAM wanted to take advantage of convening this international Panel of experts by having other related test methods reviewed. NICEATM-ICCVAM had other topics they wanted to review, so they consolidated them for one Panel at one meeting. It resulted in an aggressive agenda and the Panel was very thorough. They took their time to do a careful, comprehensive review that in the long-term would benefit the entire project.

C. SACATM Discussion

Dr. Freeman asked Dr. Levine about EPA's notice of proposed rule making for GHS adoption and whether EPA would adopt the GHS classification system. Dr. Levine said no decision would be made until a new Assistant Administrator is confirmed. Dr. Freeman said the Classification, Labeling and Packaging regulation in the EU system, which represents their acceptance of GHS, has been released. The EU system will merge with GHS in 2010. He said the GHS system represents the future and he was unsure what the United States is doing regarding the three scoring methods. He expressed confusion regarding the earlier conclusion for the use of BCOP to screen for

corrosives or severe irritants and the newer conclusion for its use to screen for substances not labeled as irritants using the GHS or EU system. He said it was at opposite ends of the spectrum and that if the United States were going to adopt GHS in the future, the EPA method should not matter. He expressed concern about classification of materials between those identified as severe irritants and non-irritants. Dr. Levine compared this classification to the issue with classifying skin irritation, where identification of the extremes is possible. She considered it a learning opportunity and suggested other agencies should also address this issue.

Dr. Barile, a lead discussant, agreed with Dr. Freeman. He understood from the 2006 review that both BCOP and ICE were approved for identifying corrosives and severe irritants, but in the 2009 conclusions, only BCOP was approved for the classification of corrosives and severe irritants. Dr. Stokes clarified that BCOP and ICE are still recommended for identifying corrosives and severe irritants. In the 2009 review, the recommendations for the use of ICE have not changed; ICE was not recommended for the identification of all ocular hazard categories as defined by the EPA, EU, and GHS classification systems. In addition, ICE was not recommended as a screening test to identify substances not labeled as irritants from all other hazard categories as defined by the GHS, EPA, and EU classification systems. Dr. Stokes emphasized that one of the reasons NICEATM-ICCVAM is using the term "not labeled as irritant," is that under the EU and GHS classification systems, even if a material is considered "not labeled as an irritant," it can still cause a considerable amount of irritation. For example, 43 substances not classified as irritants in the GHS or EU scheme are EPA Category III or higher. Category III substances cause lesions that persist for more than 24 hours, but clear by seven days. Dr. Stokes also noted that the IRE was not recommended because there are not enough data using all four endpoints, as in the current ICCVAM-recommended protocol. HET-CAM was proposed by ICCVAM to identify non-labeled surfactants and surfactant-containing compounds. The Panel disagreed with the ICCVAM recommendations because they considered the number of substances in the intermediate irritancy categories (i.e., mild and/or moderate irritants) to be insufficient.

Dr. Barile asked about use of the CM in ocular testing and the status of the testing, given that the machine is no longer available. Dr. Stokes said a new version of the CM is being developed that will measure additional endpoints. The new machine will need to meet or exceed the performance for the existing CM. Dr. Barile said little information had been presented on the CM as to what it tested and he asked why mouse fibroblasts were used. He suggested a more extensive review of CM by ICCVAM and more background information. Dr. Levine said the EPA has a policy of not recommending a brand or product; guidelines are based on performance standards. Dr. Barile also asked about use of the 2006 BRD database. Dr. Merrill said data from the AMCP submission were added to the BCOP database from 2006, but the available database for ICE had not changed since 2006.

Dr. Fox, a lead discussant, said he agreed with the report but had some comments on the science. The CM is an antiquated tool that is not sophisticated enough for use in ocular methods; there are better tools available. The methodology should be validated, not the instrument. He said in the original review, the BCOP was found acceptable to

detect corrosives, but has been upgraded to detect non-labeled materials. Dr. Stokes said more data had been added from the AMCP submission. BCOP was originally evaluated for its accuracy in classifying substances as either severe or non-severe, with irreversible or reversible effects, respectively. Accuracy for identifying moderate, mild, and non-labeled categories was not performed in the original review. Dr. Freeman said there was some dissension on the BCOP conclusions in 2006. Dr. Fox said that the local anesthetics recommended for use are esters, which have short half-lives; he asked why amides, which are longer acting, were not chosen for use. A disadvantage of local anesthetics is that they create tear breakup time and allow the compound increased access to the eye. He said a topical ophthalmic amide anesthetic might be a better option for pain control in the Draize test.

Dr. Karen Brown, a lead discussant, said the use of anesthetics for the Draize test was overdue. She said it should be a requirement unless there is justification for non-use. Systemic anesthesia should be used as well as topical anesthetics. She agreed with the Panel's recommendations, but asked for more information on the two AMCP testing strategies saying more work should be done in that area and it should move forward quickly. Individual tests were done with the BCOP and EO and it appeared they could differentiate severe from moderate and mild AMCPs. She asked how companies could be encouraged to generate more data for the AMCPs, similar to GlaxoSmithKline doing more research on the IRE. Dr. Stokes said the Ocular Toxicology WG's recommendation was to encourage industry to generate more data. Accordingly, the EPA just issued a proposal for a pilot project to encourage industry to generate data that would utilize the methods in the strategy. Dr. Levine said the EPA is proposing an eighteen-month pilot. Companies will provide both *in vitro* data and Draize data on similar products. The project will collect incident information on products that have been on the market for eight to ten years without labeling. The EPA will then make labeling decisions and evaluate how it is working. Dr. Karen Brown said the sequence of tests looked very promising.

Dr. Hansen, a lead discussant, concurred with the previous comments and said it was encouraging and long overdue that ICCVAM was moving toward requiring topical anesthetics and systemic analgesics.

Mr. Wnorowski, a lead discussant, said his company had developed some of the data several years ago on the anesthetics. His company has been successfully using anesthetic pretreatments for all its studies. He supported the other models moving forward and being accepted for regulatory purposes.

Dr. Freeman concurred with discussion on the use of anesthetics in the Draize test. Dr. Meyer asked how much is enough with respect to ICCVAM, and would the regulatory agencies accept a partial solution in the identification of classes II, III, and IV. Most of the pain and distress occurs with class I chemicals. She suggested moving forward rather than continuing to address the low rates of performance for the other classifications. Dr. Ehrich asked Dr. Hayes about the Panel's specific recommendation for the use of the analgesic buprenorphine. Dr. Hayes said this was based on strong recommendations from the veterinary anesthesiologist and ophthalmologists on the

Panel, based on their clinical experience. He said the important concept was to use a systemic analgesic first followed by a topical anesthetic prior to test substance application, and then to continue treatment with systemic analgesics as long as necessary.

Dr. Charles said harmonization is needed for assessing the performance criteria for the assays from a drug development perspective. Once there is harmonization, there is a need for guidance and strategy. He suggested assessing the other methods in a similar fashion to the AMCPs, and categorizing the test article based on a multiple assay strategy as opposed to doing more work on each individual assay. Dr. Stokes said an ECVAM-sponsored workshop suggested a top-down, bottom-up approach using a three-category system. An *in vitro* test or battery of tests would be needed that could identify all substances that could cause irreversible effects (i.e., all category I substances, with a high degree of certainty). All other categories would involve reversible damage and not cause permanent effects. Another test or battery would then be used only to identify substances that do not cause significant irritation (i.e., non-labeled substances). It would not require a high degree of sensitivity, but would identify most substances in this category without significant over-labeling. All other substances would be classified as mild or moderate. Further testing could be done to differentiate mild and moderate substances yielding a lower hazard warning for mild substances. This top-down, bottom-up approach is being pursued for both dermal and ocular irritation. Dr. Stokes explained that for ocular testing the methods are not available for the top or bottom for the level of performance needed. Not enough data are currently available to support a completely non-animal approach. Dr. Freeman said it was debatable because the BCOP could identify the highs and lows using GHS. Dr. Stokes said there are significant restrictions on categories of substances for which BCOP can be used, as some chemical classes and physical properties result in significant false negative results, which would not be acceptable in a top-down decision model.

Dr. Nicolaysen asked why the Panel recommended the dose of 0.01 mg/kg buprenorphine, which is lower than the 0.05 mg/kg used clinically. Dr. Hayes said the dose was based on clinical experience. Dr. Nicolaysen said there should be better evidence to use the lower dose. Dr. Marilyn Brown expressed some concern about the handling-stress induced in animals with the administration of the both analgesics and anesthetics, but with differing dosing schedules. Dr. Corcoran said there did not appear to be a consensus standard of care. He thought the recommendations to be overly proscriptive and suggested establishing an expectation for care with the goal of relieving pain with an antinociceptive and an anesthetic at appropriate doses and dosage schedules.

This page intentionally left blank

Appendix F

Relevant U.S. Federal and International Ocular Toxicity Regulations, Labeling, and Test Guidelines

This page intentionally left blank

Appendix F1

Table of Relevant U.S. Federal and International Ocular Testing Regulations for Hazard Classification and Labeling

Note to the Reader:
Regulations may be updated in the future. It is recommended that users review the most current version of all regulations identified.

Electronic versions of United States Code (U.S.C.) can be obtained at:
http://www.gpoaccess.gov/uscode/index.html

Electronic versions of the Code of Federal Regulations (CFR) can be obtained at:
http://www.gpoaccess.gov/cfr/index.html

This page intentionally left blank

Eye Irritation/Corrosion Testing: Relevant U.S. Federal Laws, Regulations, Guidelines, and Recommendations				
Agency, Center, or Office	**Regulated Products**	**Statutory Requirements**	**Regulations (Applications)**	**Guidelines and Recommendations**
CPSC	Consumer Products	Federal Hazardous Substances Act (U.S.C. Title 15, Chapter 47)	16 CFR 1500.3 (Definitions) 16 CFR 1500.42 (Test for Eye Irritants) 16 CFR 1500.121 (Labeling)	Animal Testing Policy (1984)
EPA/OPPTS	Chemicals as defined by the Toxic Substances Control Act Pesticides	Toxic Substances Control Act (U.S.C. Title 15, Chapter 53) Federal Insecticide, Fungicide, and Rodenticide Act (U.S.C. Title 7, Chapter 6)	40 CFR 716 (Safety Data) 40 CFR 717 (Adverse Reactions) 40 CFR 720 (Premanufacture Notification) 40 CFR 156 (Labeling) 40 CFR 158 (Pesticide Data)	OPPTS 870.2400 (1998)[1] Label Review Manual (2003)[2]

continued

[1] See **Appendix F2.**
[2] Available at: http://www.epa.gov/oppfead1/labeling/lrm/.

Eye Irritation/Corrosion Testing: Relevant U.S. Federal Laws, Regulations, Guidelines, and Recommendations (continued)				
Agency, Center, or Office	**Regulated Products**	**Statutory Requirements**	**Regulations (Applications)**	**Guidelines and Recommendations**
FDA/CFSAN FDA/CDER	Cosmetics[3] Pharmaceuticals	Federal Food, Drug, and Cosmetic Act (U.S.C. Title 21, Chapter 9) Public Health Service Act (U.S.C. Title 42, Chapter 6A)	21 CFR 70 (Color additives in food, medical devices, and cosmetics) 21 CFR 312 (IND Application) 21 CFR 314 (IND Approval) 21 CFR 701 (Cosmetic Labeling) 21 CFR 740 (Cosmetic Warning Statement)	No Specific Guidelines or Recommendations on Eye Irritation/Corrosion Testing Are Provided.
OSHA	Chemicals	Occupational Safety and Health Act of 1970 (U.S.C. Title 29, Chapter 15)	29 CFR 1910.1200 (Hazard Communication Standard) 16 CFR 1500.42 (Test for Eye Irritants)	No Specific Guidelines or Recommendations on Eye Irritation/Corrosion Testing Are Provided.

[3] FDA does not have authority for pre-market approval of cosmetics or cosmetic ingredients with the exception of color additives. However, the FDA may enforce action against products or ingredients that are in violation of Federal labeling laws, including provision of adequate safety information.

Relevant Ocular Testing Regulations for Hazard Classification and Labeling: European Union	
Regulated Products	**Regulations and Directives**
Substances and Mixtures	Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 (CLP, Classification Labelling and Packaging), amending and repealing Directives 67/548/EEC (DSD, Dangerous Substances Directive) and 1999/45/EC (DPD, Dangerous Preparations Directive), and amending Regulation (EC) No 1907/2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 (REACH, Registration, Evaluation, Authorisation and Restriction of Chemicals)
Plant Protection Products	Council Directive 91/414/EEC of 15 July 1991 as amended
Cosmetics	Council Directive 76/768/EEC of 27 July 1976 as amended
Biocidal Products	Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 as amended

Relevant Ocular Testing Regulations for Hazard Classification and Labeling: United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS)	
Scope	**Legal Instruments and Recommendations**
Chemicals (Substances and Mixtures)	Globally Harmonized System of Classification and Labelling of Chemicals (UN 2007), Part 3, Chapter 3.2.4 (Serious eye damage/eye irritation)

This page intentionally left blank

Appendix F2

EPA OPPTS Guidance Document 870.2400 (August 1998)

This page intentionally left blank

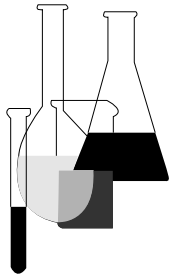
United States
Environmental Protection
Agency

Prevention, Pesticides
and Toxic Substances
(7101)

EPA 712–C–98–195
August 1998

EPA

Health Effects Test Guidelines OPPTS 870.2400 Acute Eye Irritation



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512–0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (http://www.epa.gov/epahome/research.htm) under the heading ''Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines.''

OPPTS 870.2400 Acute eye irritation.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are OPPTS 798.4500 Primary Eye Irritation; OPP 81–4 Acute Eye Irritation—Rabbit (Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09–82–025, 1982; and OECD 405 Acute Eye Irritation/ Corrosion.

(b) **Purpose.** (1) In the assessment and evaluation of the toxic characteristics of a substance, determination of the irritant and/or corrosive effects on eyes of mammals is an important initial step. Information derived from this test serves to indicate the existence of possible hazards likely to arise from exposure of the eyes and associated mucous membranes to the test substance.

(2) Data on primary eye irritation are required by 40 CFR 158.340 to support the registration of each manufacturing-use product and end-use product. (See § 158.50 to determine whether these data must be submitted and which purity/grade of the test substance should be tested.)

(c) **Definitions.** The definitions in section 3 of TSCA and in 40 CFR Part 792—Good Laboratory Practice Standards (GLP) apply to this test guideline. The following definitions also apply to this test guideline.

Eye corrosion is the production of irreversible tissue damage in the eye following application of a test substance to the anterior surface of the eye.

Eye irritation is the production of reversible changes in the eye following the application of a test substance to the anterior surface of the eye.

(d) **Principle of the test method.** The substance to be tested is applied in a single dose to one of the eyes in each of several experimental animals; the untreated eye is used to provide control information. The degree of irritation/corrosion is evaluated and scored at specified intervals and is fully described to provide a complete evaluation of the effects. The duration of the study should be sufficient to permit a full evaluation of the reversibility or irreversibility of the effects observed. The period of observation should be at least 72 h, but need not exceed 21 days. Animals showing severe and enduring signs of distress and pain may need to be killed in a humane fashion.

(e) **Initial considerations.** (1) Strongly acidic or alkaline substances, for example, with a demonstrated pH of 2 or less or 11.5 or greater, need not be tested owing to their predictable corrosive properties. Buffer capacity should also be taken into account.

(2) Materials which have demonstrated definite corrosion or severe irritation in a dermal study need not be further tested for eye irritation. It may be presumed that such substances will produce similarly severe effects in the eyes.

(3) Results from well validated and accepted *in vitro* test systems may serve to identify corrosives or irritants such that the test material need not be tested *in vivo*.

(f) **Test procedures**—(1) **Animal selection**—(i) **Species and strain.** A variety of experimental animals has been used, but it is recommended that testing should be performed using healthy adult albino rabbits. Commonly used laboratory strains should be used. If another mammalian species is used, the tester should provide justification/reasoning for its selection.

(ii) **Number of animals.** A single animal should be considered if marked effects are anticipated. If the results of this test in one animal suggest the test substance to be a severe irritant (reversible effect) or corrosive (irreversible effect) to the eye using the procedure described, further tests may not need to be performed. In cases other than a single animal test, at least three animals should be used. Occasionally, further testing in additional animals may be appropriate to clarify equivocal responses.

(2) **Dose level.** For testing liquids, a dose of 0.1 mL is recommended. In testing solids, pastes, and particulate substances, the amount used should have a volume of 0.1 mL, or a weight of not more than 100 mg (the weight must always be recorded). If the test material is solid or granular, it should be ground to a fine dust. The volume of particulates should be measured after gently compacting them (e.g. by tapping the measuring container). To test a substance contained in a pressurized aerosol container, the eye should be held open and the test substance administered in a single burst of about 1 sec from a distance of 10 cm directly in front of the eye. The dose may be estimated by weighing the container before and after use. Care should be taken not to damage the eye. Pump sprays should not be used but instead the liquid should be expelled and 0.1 mL collected and instilled into the eye as described for liquids. For volatile substances, the dose may be estimated by weighing the container before and after use.

(3) **Examination of eyes prior to test.** Both eyes of each experimental animal provisionally selected for testing should be examined within 24 h before testing starts by the same procedure to be used during the

test examination. Animals showing eye irritation, ocular defects, or pre-existing corneal injury should not be used.

(4) **Application of the test substance.** (i) The test substance should be placed in the conjunctival sac of one eye of each animal after gently pulling the lower lid away from the eyeball. The lids are then gently held together for about 1 sec in order to limit loss of the material. The other eye, which remains untreated, serves as a control. If it is thought that the substance may cause extreme pain, local anesthetic may be used prior to instillation of the test substance. The type and concentration of the local anesthetic should be carefully selected to ensure that no significant differences in reaction to the test substance will result from its use. The control eye should be similarly anesthetized.

(ii) The eyes of the test animals should not be washed out for 24 h following instillation of the test substance. At 24 h, a washout may be used if considered appropriate. This is to show whether washing with water palliates or exacerbates irritation.

(iii) For some substances shown to be irritating by this test, additional testing using animals with eyes washed soon after instillation of the substance may be indicated. Half a minute after instillation, the eyes of the animals are washed with water for 30 sec, using a volume and velocity of flow which will not cause injury.

(5) **Observation period.** The duration of the observation period is at least 72 h, and should not be fixed rigidly, but should be sufficient to evaluate fully the reversibility or irreversibility of the effects observed. The observation period normally need not exceed 21 days after instillation.

(6) **Clinical examination and scoring.** (i) The eyes should be examined at 1, 24, 48, and 72 h. If there is no evidence of irritation at 72 h, the study may be ended. Extended observation (e.g. at 7 and 21 days) may be necessary if there is persistent corneal involvement or other ocular irritation in order to determine the progress of the lesions and their reversibility or irreversibility. In addition to the observations of the cornea, iris and conjunctivae, any other lesions which are noted should be recorded and reported. The grades of ocular reaction using the following table should be recorded at each examination.

Grades for Ocular Lesions	
Cornea	
Opacity: Degree of density (area most dense taken for reading). No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	*1
Easily discernible translucent area, details of iris slightly obscured	*2
Nacrous area, no details or iris visible, size of pupil barely discernible	*3
Opaque cornea, iris not discernible through the opacity	*4
Iris	
Normal	0
Markedly deepened rugae, congestion, swelling moderate circumcorneal hyperemia, or injection, any of these or combination of any thereof, iris still reacting to light (sluggish reaction is positive)	*1
No reaction to light, hemorrhage, gross destruction (any or all of these)	*2
Conjunctivae	
Redness (refers to palpebral and bulbar conjunctivae, excluding cornea and iris).	
Blood vessels normal	0
Some blood vessels definitely hyperemic (injected)	1
Diffuse, crimson color, individual vessels not easily discernible	*2
Diffuse beefy red	*3
Chemosis (refers to lids and/or nictitating membranes)	
No swelling	0
Any swelling above normal (includes nictitating membranes)	1
Obvious swelling with partial eversion of lids	*2
Swelling with lids about half closed	*3
Swelling with lids more than half-closed	*4

*Starred figures indicate positive grades.

(ii) Examination of reactions can be facilitated by use of a binocular loupe, hand slit-lamp, biomicroscope, or other suitable device. After recording the observations at 24 h, the eyes of any or all rabbits may be further examined with the aid of fluorescein.

(iii) The grading of ocular responses is subject to various interpretations. To promote harmonization and to assist testing laboratories and those involved in making and interpreting the observations, an illustrated guide in grading eye irritation should be used.

(g) **Data and reporting—**(1) **Data summary.** Data should be summarized in tabular form, showing for each individual animal the irritation scores at observation time up until reversal (nonpositive grades) or 21 days when the test is concluded; a description of the degree and nature of irritation; the presence of serious lesions and any effects other than ocular which were observed.

(2) **Evaluation of the results.** The ocular irritation scores should be evaluated in conjunction with the nature and reversibility or otherwise of the responses observed. The individual scores do not represent an absolute standard for the irritant properties of a material. They should be viewed as reference values and are only meaningful when supported by a full description and evaluation of the observations.

(3) **Test report.** In addition to the reporting requirements as specified under 40 CFR part 792, subpart J, the following specific information should be reported:

(i)Species, strain, sex, age, and source of test animal.

(ii) Rationale for selection of species (if species is other than the species preferred.

(iiii) Tabulation of irritant/corrosive response data for each individual animal at each observation time point (e.g. 1, 24, 48, and 72 h until reversibility of lesions or termination of the test).

(iv) Description of any lesions observed.

(v) Narrative description of the degree and nature of irritation or corrosion observed.

(vi) Description of the method used to score the irritation at 1, 24, 48, and 72 h (e.g. hand slit-lamp, biomicroscope, fluorescein stain).

(vii) Description of any nonocular effects noted.

(viii) Description of any pre-test conditioning, including diet, quarantine, and treatment of disease.

(ix) Description of caging conditions including number (and any change in number) of animals per cage, bedding material, ambient temperature and humidity, photoperiod, and identification of diet of test animal.

(x) Manufacturer, source, purity, and lot number of test substance.

(xi) Physical nature, and, where appropriate, concentration and pH value for the test substance.

(xii) Identification, composition, and characteristics of any vehicles (e.g., diluents, suspending agents, emulsifiers, and anesthetics) or other materials used in administering the test substance.

(xiii) A list of references cited in the body of the report, i.e., references to any published literature used in developing the test protocol, performing the testing, making and interpreting observations, and compiling and evaluating the results.

(h) **References.** The following references should be consulted for additional background information on this test guideline

(1) Buehler, E.V. and Newmann, E.A. A Comparison of Eye Irritation in Monkeys and Rabbits. *Toxicology and Applied Pharmacology* 6:701–710 (1964).

5

(2) Draize, J.H. *Dermal Toxicity. Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics.* The Association of Food and Drug Officials of the United States (1959) 3rd printing 1975, pp. 49–52.

(3) Draize, J.H. et al. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *Journal of Pharmacology and Experimental Therapeutics.* 83:377–390 (1944).

(4) Loomis, T.A. *Essentials of Toxicology.* Lea and Febicer, Philadelphia 3rd ed. 1978 pp. 226–232.

(5) Kay, J.H. and Calandra, J.C., Interpretation of eye irritation tests. *Journal of the Society of Cosmetic Chemists* 13:281–289 (1962).

(6) National Academy of Sciences. *Principles and Procedures for Evaluating the Toxicity of Household Substances.* A report propared by the Committee for the revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).

(7) World Health Organization. *Part I. Environmental Health Criteria 6. Principles and Methods for Evaluating the Toxicity of Chemicals.* World Health Organization, Geneva (1978).

Appendix F3

EPA Office of Pesticide Programs Label Review Manual (August 2003)

Electronic versions of the EPA LRM can be obtained at:
http://www.epa.gov/oppfead1/labeling/lrm/

This page intentionally left blank

Appendix F4

Organisation for Economic Co-operation and Development (OECD) Test Guideline 405 (Adopted April 2002)

This page intentionally left blank

OECD/OCDE **405**

Adopted:
24th April 2002

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Acute Eye Irritation/Corrosion

INTRODUCTION

1. OECD Guidelines for Testing of Chemicals are periodically reviewed to ensure that they reflect the best available science. In the review of this Guideline, special attention was given to possible improvements through the evaluation of all existing information on the test substance in order to avoid unnecessary testing in laboratory animals and thereby address animal welfare concerns. This updated version of Guideline 405 (adopted in 1981 and first revised in 1987) includes the recommendation that prior to undertaking the described *in vivo* test for acute eye irritation/corrosion, a weight-of-the-evidence analysis be performed (1) on the existing relevant data. Where insufficient data are available, it is recommended that they be developed through application of sequential testing (2)(3). The testing strategy includes the performance of validated and accepted *in vitro* tests and is provided as a Supplement to the Guideline. In addition, the use of an *in vivo* dermal irritation/corrosion test to predict eye corrosion prior to consideration of an *in vivo* eye test is recommended in this Guideline.

2. Definitions of acute eye irritation and corrosion are set out in the Annex to the Guideline.

INITIAL CONSIDERATIONS

3. In the interest of both sound science and animal welfare, *in vivo* testing should not be considered until all available data relevant to the potential eye corrosivity/irritation of the substance has been evaluated in a weight-of-the-evidence analysis. Such data will include evidence from existing studies in humans and/or laboratory animals, evidence of corrosivity/irritation of one or more structurally related substances or mixtures of such substances, data demonstrating high acidity or alkalinity of the substance (4)(5), and results from validated and accepted *in vitro* or *ex vivo* tests for skin corrosion and irritation (6)(7). The studies may have been conducted prior to, or as a result of, a weight-of-the-evidence analysis.

4. For certain substances, such an analysis may indicate the need for *in vivo* studies of the ocular corrosion/irritation potential of the substance. In all such cases, before considering the use of the *in vivo* eye test, preferably a study of the *in vivo* dermal effects of the substance should be conducted first and evaluated in accordance with Testing Guideline 404 (8). The application of a weight-of-the-evidence analysis and the sequential testing strategy should decrease the need for *in vivo* testing for eye corrosivity/irritation of substances for which sufficient evidence already exists from other studies. If a determination of eye corrosion or irritation potential cannot be made using the sequential testing strategy, even after the performance of an *in vivo* study of dermal corrosion and irritation, an *in vivo* eye corrosion/irritation test may be performed.

5. A preferred sequential testing strategy, which includes the performance of validated *in vitro* or *ex vivo* tests for corrosion/irritation, is included as a Supplement to this guideline. The strategy was developed at, and unanimously recommended by the participants of, an OECD workshop (9), and has been adopted as the recommended testing strategy in the Globally Harmonised System for the Classification of Chemical Substances (GHS) (10). It is recommended that this testing strategy be followed prior to undertaking *in vivo* testing. For new substances it is the recommended stepwise testing approach for

developing scientifically sound data on the corrosivity/irritation of the substance. For existing substances with insufficient data on skin and eye corrosion/irritation, the strategy should be used to fill missing data gaps. The use of a different testing strategy or procedure, or the decision not to use a stepwise testing approach, should be justified.

PRINCIPLE OF THE *IN VIVO* TEST

6. The substance to be tested is applied in a single dose to one of the eyes of the experimental animal; the untreated eye serves as the control. The degree of eye irritation/corrosion is evaluated by scoring lesions of conjunctiva, cornea, and iris, at specific intervals. Other effects in the eye and adverse systemic effects are also described to provide a complete evaluation of the effects. The duration of the study should be sufficient to evaluate the reversibility or irreversibility of the effects.

7. Animals showing continuing signs of severe distress and/or pain at any stage of the test should be humanely killed, and the substance assessed accordingly. Criteria for making the decision to humanely kill moribund and severely suffering animals are the subject of a separate Guidance Document (11).

PREPARATIONS FOR THE *IN VIVO* TEST

Selection of species

8. The albino rabbit is the preferable laboratory animal, and healthy young adult animals are used. A rationale for using other strains or species should be provided.

Preparation of animals

9. Both eyes of each experimental animal provisionally selected for testing should be examined within 24 hours before testing starts. Animals showing eye irritation, ocular defects, or pre-existing corneal injury should not be used.

Housing and feeding conditions

10. Animals should be individually housed. The temperature of the experimental animal room should be 20°C (± 3°C) for rabbits. Although the relative humidity should be at least 30% and preferably not exceed 70%, other than during room cleaning, the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unrestricted supply of drinking water.

TEST PROCEDURE

Application of the test substance

11. The test substance should be placed in the conjunctival sac of one eye of each animal after gently pulling the lower lid away from the eyeball. The lids are then gently held together for about one second in order to prevent loss of the material. The other eye, which remains untreated, serves as a control.

Irrigation

12. The eyes of the test animals should not be washed for at least 24 hours following instillation of the test substance, except for solids (see paragraph 16), and in case of immediate corrosive or irritating effects. At 24 hours a washout may be used if considered appropriate.

13. Use of a satellite group of animals to investigate the influence of washing is not recommended unless it is scientifically justified. If a satellite group is needed, two rabbits should be used. Conditions of washing should be carefully documented, e.g., time of washing; composition and temperature of wash solution; duration, volume, and velocity of application.

Dose level

(1) Testing of liquids

14. For testing liquids, a dose of 0.1 mL is used. Pump sprays should not be used for instilling the substance directly into the eye. The liquid spray should be expelled and collected in a container prior to instilling 0.1 mL into the eye.

(2) Testing of solids

15. When testing solids, pastes, and particulate substances, the amount used should have a volume of 0.1 mL or a weight of not more than 100 mg. The test material should be ground to a fine dust. The volume of solid material should be measured after gently compacting it, e.g., by tapping the measuring container. If the solid test substance has not been removed from the eye of the test animal by physiological mechanisms at the first observation time point of 1 hour after treatment, the eye may be rinsed with saline or distilled water.

(3) Testing of aerosols

16. It is recommended that all pump sprays and aerosols be collected prior to installation into the eye. The one exception is for substances in pressurised aerosol containers, which cannot be collected due to vaporisation. In such cases, the eye should be held open, and the test substance administered to the eye in a simple burst of about one second, from a distance of 10 cm directly in front of the eye. This distance may vary depending on the pressure of the spray and its contents. Care should be taken not to damage the eye from the pressure of the spray. In appropriate cases, there may be a need to evaluate the potential for "mechanical" damage to the eye from the force of the spray.

17. An estimate of the dose from an aerosol can be made by simulating the test as follows: the substance is sprayed on to weighing paper through an opening the size of a rabbit eye placed directly before the paper. The weight increase of the paper is used to approximate the amount sprayed into the eye. For volatile substances, the dose may be estimated by weighing a receiving container before and after removal of the test material.

Initial test (*in vivo* eye irritation/corrosion test using one animal)

18. As articulated in the sequential testing strategy (Supplement to Guideline), it is strongly recommended that the *in vivo* test be performed initially using one animal.

19. If the results of this test indicate the substance to be corrosive or a severe irritant to the eye using the procedure described, further testing for ocular irritancy should not be performed.

Local anaesthetics

20. Local anaesthetics may be used on a case-by-case basis. If the weight-of-the-evidence analysis indicates that the substance has the potential to cause pain, or initial testing shows that a painful reaction will occur, a local anaesthetic may be used prior to instillation of the test substance. The type, concentration, and dose of the local anaesthetic should be carefully selected to ensure that differences in reaction to the test substance will not result from its use. The control eye should be similarly anaesthetised.

Confirmatory test (*in vivo* eye irritation test with additional animals)

21. If a corrosive effect is not observed in the initial test, the irritant or negative response should be confirmed using up to two additional animals. If a severe irritant effect is observed in the initial test indicating a possible strong (irreversible) effect in the confirmatory testing, it is recommended that the confirmatory test be conducted in a sequential manner in one animal at a time, rather than exposing the two additional animals simultaneously. If the second animal reveals corrosive or severe irritant effects, the test is not continued. Additional animals may be needed to confirm weak or moderate irritant responses.

Observation period

22. The duration of the observation period should be sufficient to evaluate fully the magnitude and reversibility of the effects observed. However, the experiment should be terminated at any time that the animal shows continuing signs of severe pain or distress (9). To determine reversibility of effects, the animals should be observed normally for 21 days post administration of the test substance. If reversibility is seen before 21 days, the experiment should be terminated at that time.

Clinical observations and grading of eye reactions

23. The eyes should be examined at 1, 24, 48, and 72 hours after test substance application. Animals should be kept on test no longer than necessary once definitive information has been obtained. Animals showing continuing severe pain or distress should be humanely killed without delay, and the substance assessed accordingly. Animals with the following eye lesions post-instillation should be humanely killed: corneal perforation or significant corneal ulceration including staphyloma; blood in the anterior chamber of the eye; grade 4 corneal opacity which persists for 48 hours; absence of a light reflex (iridial response grade 2) which persists for 72 hours; ulceration of the conjunctival membrane; necrosis of the conjuctivae or nictitating membrane; or sloughing. This is because such lesions generally are not reversible.

24. Animals that do not develop ocular lesions may be terminated not earlier than 3 days post instillation. Animals with mild to moderate lesions should be observed until the lesions clear, or for 21 days, at which time the study is terminated. Observations should be performed at 7, 14, and 21 days in order to determine the status of the lesions, and their reversibility or irreversibility.

25. The grades of ocular reaction (conjunctivae, cornea and iris) should be recorded at each examination (Table I). Any other lesions in the eye (e.g. pannus, staining) or adverse systemic effects should also be reported.

26. Examination of reactions can be facilitated by use of a binocular loupe, hand slit-lamp, biomicroscope, or other suitable device. After recording the observations at 24 hours, the eyes may be further examined with the aid of fluorescein.

27. The grading of ocular responses is necessarily subjective. To promote harmonisation of grading of ocular response and to assist testing laboratories and those involved in making and interpreting the observations, the personnel performing the observations need to be adequately trained in the scoring system used.

DATA AND REPORTING

Evaluation of results

28. The ocular irritation scores should be evaluated in conjunction with the nature and severity of lesions, and their reversibility or lack of reversibility. The individual scores do not represent an absolute standard for the irritant properties of a material, as other effects of the test material are also evaluated. Instead, individual scores should be viewed as reference values and are only meaningful when supported by a full description and evaluation of all observations.

Test report

29. The test report must include the following information:

Rationale for *in vivo* testing: weight-of-the-evidence analysis of pre-existing test data, including results from sequential testing strategy:

- description of relevant data available from prior testing;
- data derived in each step of testing strategy;
- description of *in vitro* tests performed, including details of procedures, results obtained with test/reference substances;
- description of *in vivo* dermal irritation / corrosion study performed, including results obtained;
- weight-of-the-evidence analysis for performing *in vivo* study

Test substance:

- identification data (e.g. CAS number, source, purity, known impurities, lot number);
- physical nature and physicochemical properties (e.g. pH, volatility, solubility, stability, reactivity with water);
- in case of a mixture, composition and relative percentages of components;
- if local anaesthetic is used, identification, purity, type, dose, and potential interaction with test substance.

Vehicle:

- identification, concentration (where appropriate), volume used;
- justification for choice of vehicle.

Test animals:

- species/strain used, rationale for using animals other than albino rabbit;
- age of each animal at start of study;
- number of animals of each sex in test and control groups (if required);

- individual animal weights at start and conclusion of test;
- source, housing conditions, diet, etc.

Results:

- description of method used to score irritation at each observation time (e.g., hand slitlamp, biomicroscope, fluorescein);
- tabulation of irritant/corrosive response data for each animal at each observation time up to removal of each animal from the test;
- narrative description of the degree and nature of irritation or corrosion observed;
- description of any other lesions observed in the eye (e.g., vascularization, pannus formation, adhesions, staining);
- description of non-ocular local and systemic adverse effects, and histopatho-ogical findings, if any.

Discussion of results.

Interpretation of the results

30. Extrapolation of the results of eye irritation studies in laboratory animals to humans is valid only to a limited degree. In many cases the albino rabbit is more sensitive than humans to ocular irritants or corrosives.

31. Care should be taken in the interpretation of data to exclude irritation resulting from secondary infection.

LITERATURE

(1) Barratt, M.D., Castell, J.V., Chamberlain, M., Combes, R.D., Dearden, J.C., Fentem, J.H., Gerner, I., Giuliani, A., Gray, T.J.B., Livingston, D.J., Provan, W.M., Rutten, F.A.J.J.L., Verhaar, H.J.M., Zbinden, P. (1995). The Integrated Use of Alternative Approaches for Predicting Toxic Hazard. ECVAM Workshop Report 8. ATLA 23, 410 - 429.

(2) de Silva, O., Cottin, M., Dami, N., Roguet, R., Catroux, P., Toufic, A., Sicard, C., Dossou, K.G., Gerner, I., Schlede, E., Spielmann, H., Gupta, K.C., Hill, R.N. (1997). Evaluation of Eye Irritation Potential: Statistical Analysis and Tier Testing Strategies. Food Chem. Toxicol 35, 159 - 164.

(3) Worth A.P. and Fentem J.H. (1999). A general approach for evaluating stepwise testing strategies ATLA 27, 161-177.

(4) Young, J.R., How, M.J., Walker, A.P., Worth W.M.H. (1988). Classification as Corrosive or Irritant to Skin of Preparations Containing Acidic or Alkaline Substance Without Testing on Animals. Toxicol. *In Vitro*, 2, 19 - 26.

(5) Neun, D.J. (1993). Effects of Alkalinity on the Eye Irritation Potential of Solutions Prepared at a Single pH. J. Toxicol. Cut. Ocular Toxicol. 12, 227 - 231.

(6) Fentem, J.H., Archer, G.E.B., Balls, M., Botham, P.A., Curren, R.D., Earl, L.K., Edsail, D.J., Holzhutter, H.G. and Liebsch, M. (1998). The ECVAM international validation study on in vitro tests for skin corrosivity. 2. Results and evaluation by the Management Team. Toxicology in Vitro 12, pp.483 – 524.

(7) EU (2000). Official Journal of The European Communities L136/91 of 8 June 2000, Method B.40 Skin Corrosion.

(8) OECD (2000). Test Guideline 404. Acute Dermal Irritation/Corrosion.

(9) OECD (1996). OECD Test Guidelines Programme: Final Report of the OECD Workshop on Harmonization of Validation and Acceptance Criteria for Alternative Toxicological Test Methods. Held in Solna, Sweden, 22 - 24 January 1996 (http://www.oecd.org/ehs/test/background.htm).

(10) OECD (1998). Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances, as endorsed by the 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, November 1998 (http://www.oecd.org/ehs/Class/HCL6.htm).

(11) OECD (2000). Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation. OECD Environmental Health and Safety Publications. Series on Testing and Assessment No. 19 (http://www.oecd.org/ehs/test/monos.htm).

405 **OECD/OCDE**

TABLE: GRADING OF OCULAR LESIONS

Cornea

Opacity: degree of density (readings should be taken from most dense area)*

No ulceration or opacity 0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre); details of iris clearly visible 1
Easily discernible translucent area; details of iris slightly obscured 2
Nacrous area; no details of iris visible; size of pupil barely discernible 3
Opaque cornea; iris not discernible through the opacity 4

Maximum possible: 4

* The area of corneal opacity should be noted

Iris

Normal 0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia; or injection; iris reactive to light (a sluggish reaction is considered to be an effect 1
Hemorrhage, gross destruction, or no reaction to light 2

Maximum possible: 2

Conjunctivae

Redness (refers to palpebral and bulbar conjunctivae; excluding cornea and iris)

Normal 0
Some blood vessels hyperaemic (injected) 1
Diffuse, crimson colour; individual vessels not easily discernible 2
Diffuse beefy red 3

Maximum possible: 3

Chemosis

Swelling (refers to lids and/or nictating membranes)

Normal 0
Some swelling above norma 1
Obvious swelling, with partial eversion of lids 2
Swelling, with lids about half closed 3
Swelling, with lids more than half closed 4

Maximum possible: 4

OECD/OCDE **405**

ANNEX

DEFINITIONS

1. Eye irritation is the production of changes in the eye following the application of a test substance to the anterior surface of the eye, which are fully reversible within 21 days of application.

2. Eye corrosion is the production of tissue damage in the eye, or serious physical decay of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application.

405 OECD/OCDE

SUPPLEMENT TO TEST GUIDELINE 405

A Sequential Testing Strategy for Eye Irritation and Corrosion

GENERAL CONSIDERATIONS

1. In the interests of sound science and animal welfare, it is important to avoid the unnecessary use of animals, and to minimise testing that is likely to produce severe responses in animals. All information on a substance relevant to its potential ocular irritation/corrosivity should be evaluated prior to considering *in vivo* testing. Sufficient evidence may already exist to classify a test substance as to its eye irritation or corrosion potential without the need to conduct testing in laboratory animals. Therefore, utilizing a weight-of-the-evidence analysis and sequential testing strategy will minimise the need for *in vivo* testing, especially if the substance is likely to produce severe reactions.

2. It is recommended that a weight-of-the-evidence analysis be used to evaluate existing information pertaining to eye irritation and corrosion of substances and to determine whether additional studies, other than *in vivo* eye studies, should be performed to help characterise such potential. Where further studies are needed, it is recommended that the sequential testing strategy be utilised to develop the relevant experimental data. For substances which have no testing history, the sequential testing strategy should be utilised to develop the data are needed to evaluate its eye corrosion/irritation. The testing strategy described in this Supplement was developed at an OECD workshop (1). It was subsequently affirmed and expanded in the Harmonised Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances, as endorsed by the 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, in November 1998 (2).

3. Although this testing strategy is not an integrated part of Test Guideline 405, it expresses the recommended approach for the determination of eye irritation/corrosion properties. This approach represents both best practice and an ethical benchmark for *in vivo* testing for eye irritation/corrosion. The Guideline provides guidance for the conduct of the *in vivo* test and summarises the factors that should be addressed before considering such a test. The sequential testing strategy provides a weight-of-the-evidence approach for the evaluation of existing data on the eye irritation/corrosion properties of substances and a tiered approach for the generation of relevant data on substances for which additional studies are needed or for which no studies have been performed. The strategy includes the performance first of validated and accepted *in vitro* or *ex vivo* tests and then of Guideline 404 skin irritation/corrosion studies under specific circumstances (3)(4).

DESCRIPTION OF THE STEPWISE TESTING STRATEGY

4. Prior to undertaking tests as part of the sequential testing strategy (Figure), all available information should be evaluated to determine the need for *in vivo* eye testing. Although significant information might be gained from the evaluation of single parameters (e.g., extreme pH), the totality of existing information should be assessed. All relevant data on the effects of the substance in question, and its structural analogues, should be evaluated in making a weight-of-the-evidence decision, and a rationale for the decision should be presented. Primary emphasis should be placed upon existing human and animal data on the substance, followed by the outcome of *in vitro* or *ex vivo* testing. *In vivo* studies of corrosive substances should be avoided whenever possible. The factors considered in the testing strategy include:

5. Evaluation of existing human and animal data (Step 1). Existing human data, e.g. clinical and occupational studies, and case reports, and/or animal test data from ocular studies should be considered first, because they provide information directly related to effects on the eyes. Thereafter, available data from human and/or animal studies investigating dermal corrosion/irritation should be evaluated. Substances with known corrosivity or severe irritancy to the eye should not be instilled into the eyes of animals, nor should substances showing corrosive or severe irritant effects to the skin; such substances should be considered to be corrosive and/or irritating to the eyes as well. Substances with sufficient evidence of non-corrosivity and non-irritancy from previously performed ocular studies should also not be tested in *in vivo* eye studies.

6. Analysis of structure activity relationships (SAR) (Step 2). The results of testing of structurally related chemicals should be considered, if available. When sufficient human and/or animal data are available on structurally related substances or mixtures of such substances to indicate their eye corrrosion/irritancy potential, it can be presumed that the test substance will produce the same responses. In those cases, the substance may not need to be tested. Negative data from studies of structurally related substances or mixtures of such substances do not constitute sufficient evidence of non-corrosivity/non-irritancy of a substance under the sequential testing strategy. Validated and accepted SAR approaches should be used to identify the corrosion and irritation potential for both dermal and ocular effects.

7. Physicochemical properties and chemical reactivity (Step 3). Substances exhibiting pH extremes such as ≤2.0 or ≥11.5 may have strong local effects. If extreme pH is the basis for identifying a substance as corrosive or irritant to the eye, then its acid/alkaline reserve (buffering capacity) may also be taken into consideration (5)(6). If the buffering capacity suggests that a substance may not be corrosive to the eye, then further testing should be undertaken to confirm this, preferably by the use of a validated and accepted *in vitro* or *ex vivo* test (see paragraph 9).

8. Consideration of other existing information (Step 4). All available information on systemic toxicity via the dermal route should be evaluated at this stage. The acute dermal toxicity of the test substance should also be considered. If the test substance has been shown to be highly toxic by the dermal route, it may not need to be tested in the eye. Although there is not necessarily a relationship between acute dermal toxicity and eye irritation/corrosion, it can be assumed that if an agent is highly toxic via the dermal route, it will also exhibit high toxicity when instilled into the eye. Such data may also be considered between Steps 2 and 3.

9. Results from *in vitro* or *ex vivo* tests (Steps 5 and 6). Substances that have demonstrated corrosive or severe irritant properties in an *in vitro* or *ex vivo* test (7)(8) that has been validated and accepted for the assessment specifically of eye or skin corrosivity/irritation, need not be tested in animals. It can be presumed that such substances will produce similar severe effects *in vivo*. If validated and accepted *in vitro/ex vivo* tests are not available, one should bypass Steps 5 and 6 and proceed directly to Step 7.

10. Assessment of *in vivo* dermal irritancy or corrosivity of the substance (Step 7). When insufficient evidence exists with which to perform a conclusive weight-of-the-evidence analysis of the potential eye irritation/corrosivity of a substance based upon data from the studies listed above, the *in vivo* skin irritation/corrosion potential should be evaluated first, using Guideline 404 (4) and the accompanying Supplement (9). If the substance is shown to produce corrosion or severe skin irritation, it should be considered to be a corrosive eye irritant unless other information supports an alternative conclusion. Thus, an *in vivo* eye test would not need to be performed. If the substance is not corrosive or severely irritating to the skin, an *in vivo* eye test should be performed.

11. *In vivo* test in rabbits (Steps 8 and 9): *In vivo* ocular testing should begin with an initial test using one animal. If the results of this test indicate the substance to be a severe irritant or corrosive to the eyes, further testing should not be performed. If that test does not reveal any corrosive or severe irritant effects, a confirmatory test is conducted with two additional animals. Depending upon the results of the confirmatory test, further tests may be needed. [see Test Guideline 405 (10)]

LITERATURE

(1) OECD (1996) OECD Test Guidelines Programme: Final Report of the OECD Workshop on Harmonization of Validation and Acceptance Criteria for Alternative Toxicological Test Methods. Held in Solna, Sweden, 22 - 24 January 1996 (http://www1.oecd.org/ehs/test/background.htm).

(2) OECD (1998) Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances, as endorsed by the 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, November 1998 (http://www1.oecd.org/ehs/Class/HCL6.htm).

(3) Worth, A.P. and Fentem J.H. (1999). A General Approach for Evaluating Stepwise Testing Strategies. ATLA 27, 161-177.

(4) OECD (2002) Guideline 404. Acute Dermal Irritation/Corrosion.

(5) Young, J.R., How, M.J., Walker, A.P., Worth W.M.H. (1988) Classification as Corrosive or Irritant to Skin of Preparations Containing Acidic or Alkaline Substance Without Testing on Animals. Toxicol. *In Vitro*, 2, 19 - 26.

(6) Fentem, J.H., Archer, G.E.B., Balls, M., Botham, P.A., Curren, R.D., Earl, L.K., Edsail, D.J., Holzhutter, H.G. and Liebsch, M. (1998) The ECVAM international validation study on in vitro tests for skin corrosivity. 2. Results and evaluation by the Management Team. Toxicology in Vitro 12, pp.483 – 524.

(7) Neun, D.J. (1993) Effects of Alkalinity on the Eye Irritation Potential of Solutions Prepared at a Single pH. J. Toxicol. Cut. Ocular Toxicol. 12, 227 - 231.

(8) EU (2002) Official Journal of The European Communities L136/91 of 8 June 2000, Method B.40 Skin Corrosion

(9) OECD (2001) Supplement to Test Guideline 404: A Sequential Testing Strategy for Skin Irritation and Corrosion.

(10) OECD (2001) Guideline 405. Acute Eye Irritation/Corrosion

OECD/OCDE **405**

FIGURE

TESTING AND EVALUATION STRATEGY FOR EYE IRRITATION/CORROSION

	Activity	Finding	Conclusion
1	Existing human and/or animal data showing effects on eyes	Severe damage to eyes	Apical endpoint; consider corrosive to eyes. No testing is needed.
		Eye irritant	Apical endpoint; consider irritating to eyes. No testing is needed.
		Not corrosive/not irritating to eyes	Apical endpoint; considered non-corrosive and non-irritating to eyes. No testing required.
	Existing human and/or animal data showing corrosive effects on skin	Skin corrosive	Assume corrosivity to eyes. No testing is needed.
	Existing human and/or animal data showing severe irritant effects on skin	Severe skin irritant	Assume irritating to eyes. No testing is needed
	↓ *no information available, or available information is not conclusive* ↓		
2	Perform SAR for eye corrosion/irritation	Predict severe damage to eyes	Assume corrosivity to eyes. No testing is needed.
		Predict irritation to eyes	Assume irritating to eyes. No testing is needed.
	Perform SAR for skin corrosion	Predict skin corrosivity	Assume corrosivity to eyes. No testing is needed.
	↓ *No predictions can be made, or predictions are not conclusive or negative* ↓		
3	Measure pH (buffering capacity, if relevant)	pH ≤ 2 or ≥ 11.5 (with high buffering capacity, if relevant)	Assume corrosivity to eyes. No testing is needed.
	↓ *2< pH < 11.5, or pH≤ 2.0 or ≥ 11.5 with low/no buffering capacity, if relevant* ↓		

405 **OECD/OCDE**

4	Evaluate systemic toxicity via the dermal route	Highly toxic at concentrations that would be tested in the eye.	Substance would be too toxic for testing. No testing is needed.
	↓ *Such information is not available, or substance is not highly toxic* ↓		
5	Perform validated and accepted *in vitro* or *ex vivo* test for eye corrosion	Corrosive response	Assume corrosivity to eyes. No further testing is needed.
	↓ *Substance is not corrosive, or internationally validated in vitro or ex vivo testing methods for eye corrosion are not yet available* ↓		
6	Perform validated and accepted *in vitro* or *ex vivo* test for eye irritation	Irritant response	Assume irritancy to eyes. No further testing is needed.
	↓ *Substance is not an irritant, or internationally validated in vitro or ex vivo testing methods for eye irritation are not yet available* ↓		
7	Experimentally assess *in vivo* skin irritation/corrosion potential (see OECD Guideline 404)	Corrosive or severe irritant response	Assume corrosivity to eyes. No further testing is needed.
	↓ *Substance is not corrosive or severely irritating to skin* ↓		
8	Perform initial *in vivo* rabbit eye test using one animal	Severe damage to eyes	Consider corrosive to eyes. No further testing is needed.
	↓ *No severe damage, or no response* ↓		
9	Perform confirmatory test using one or two additional animals	Corrosive or irritating	Consider corrosive or irritating to eyes. No further testing is needed
		Not corrosive or irritating	Consider non-irritating and non-corrosive to eyes. No further testing is needed.

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health